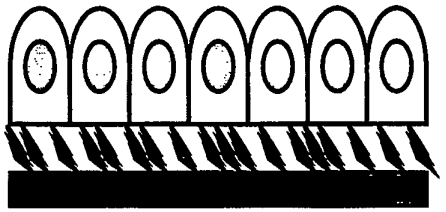
FIG.1A

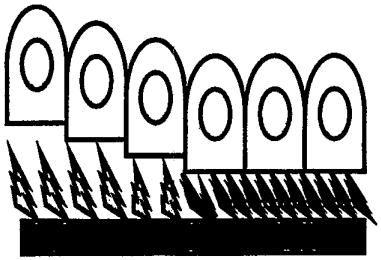
Cell Sheet Constructs

A



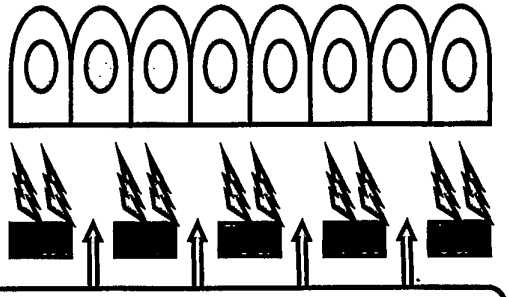
Hydrophobic PIPAAm
Remains attached: 37°C

B



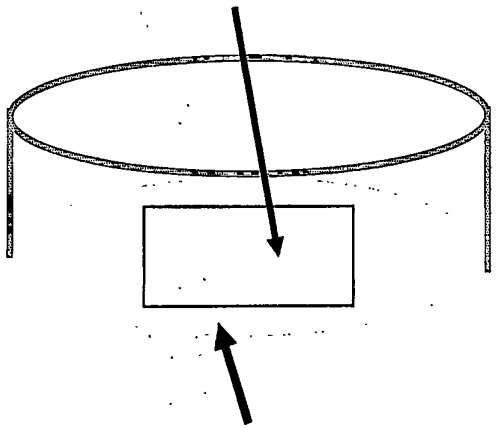
Intermediate stage
Partial detachment: 20°C

C



Hydrophilic PIPPAm
Completely detached sheet

Temperature responsive polymer
Poly (N-isopropylacrylamide) (PIPAAm)



Poly (acrylamide) (PAAm)

Exemplary Dimensions:

$1.11 \pm 0.05 cm^2$ in area

$50.2 \pm 6.0 \mu m$ thick

FIG.1B

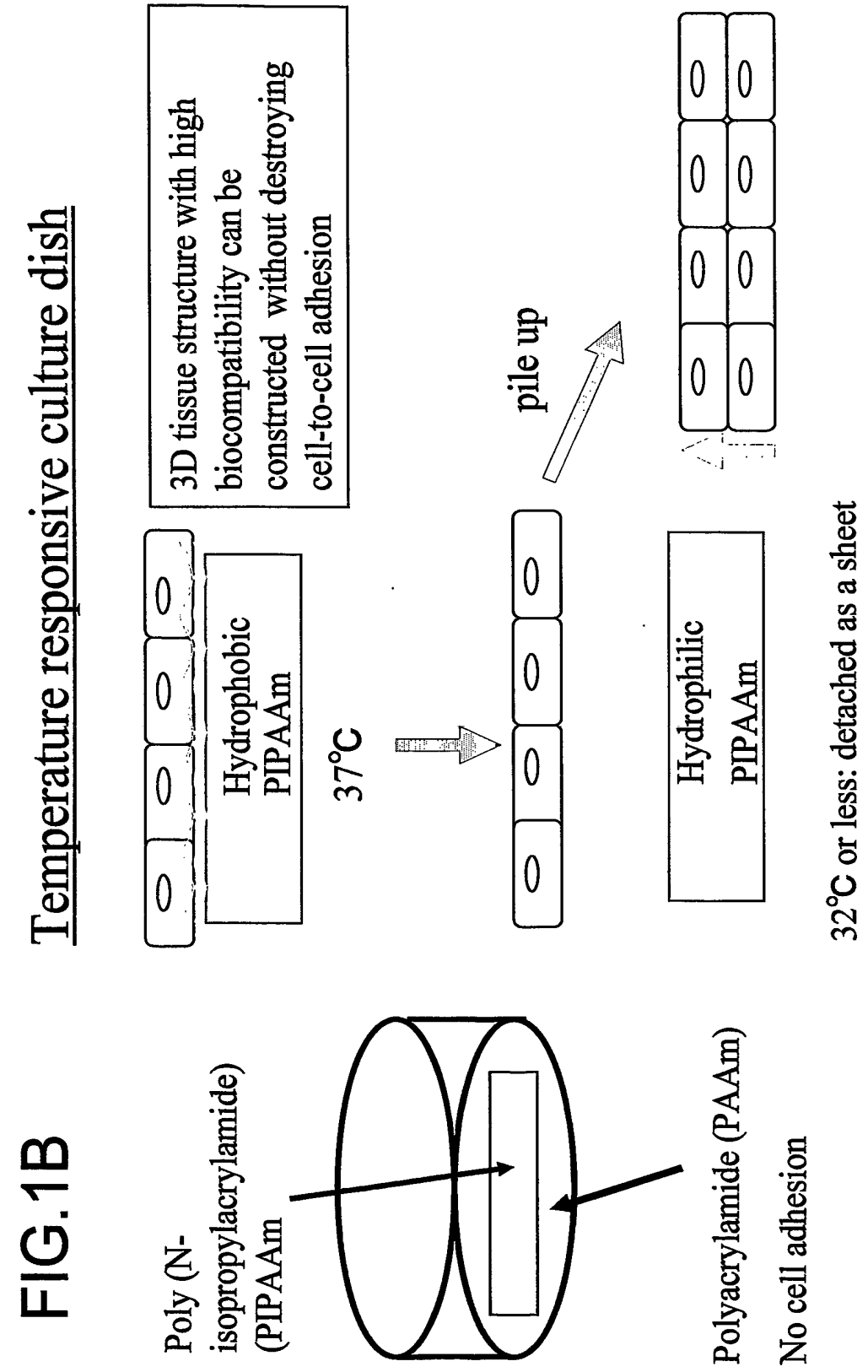

Temperature responsive culture dish
Poly (N-isopropylacrylamide) (PIPAAm
Hydrophobic PIPAAm
37°C
3D tissue structure with high biocompatibility can be constructed without destroying cell-to-cell adhesion
pile up
Hydrophilic PIPAAm
Polyacrylamide (PAAm)
No cell adhesion
32°C or less: detached as a sheet

FIG.2

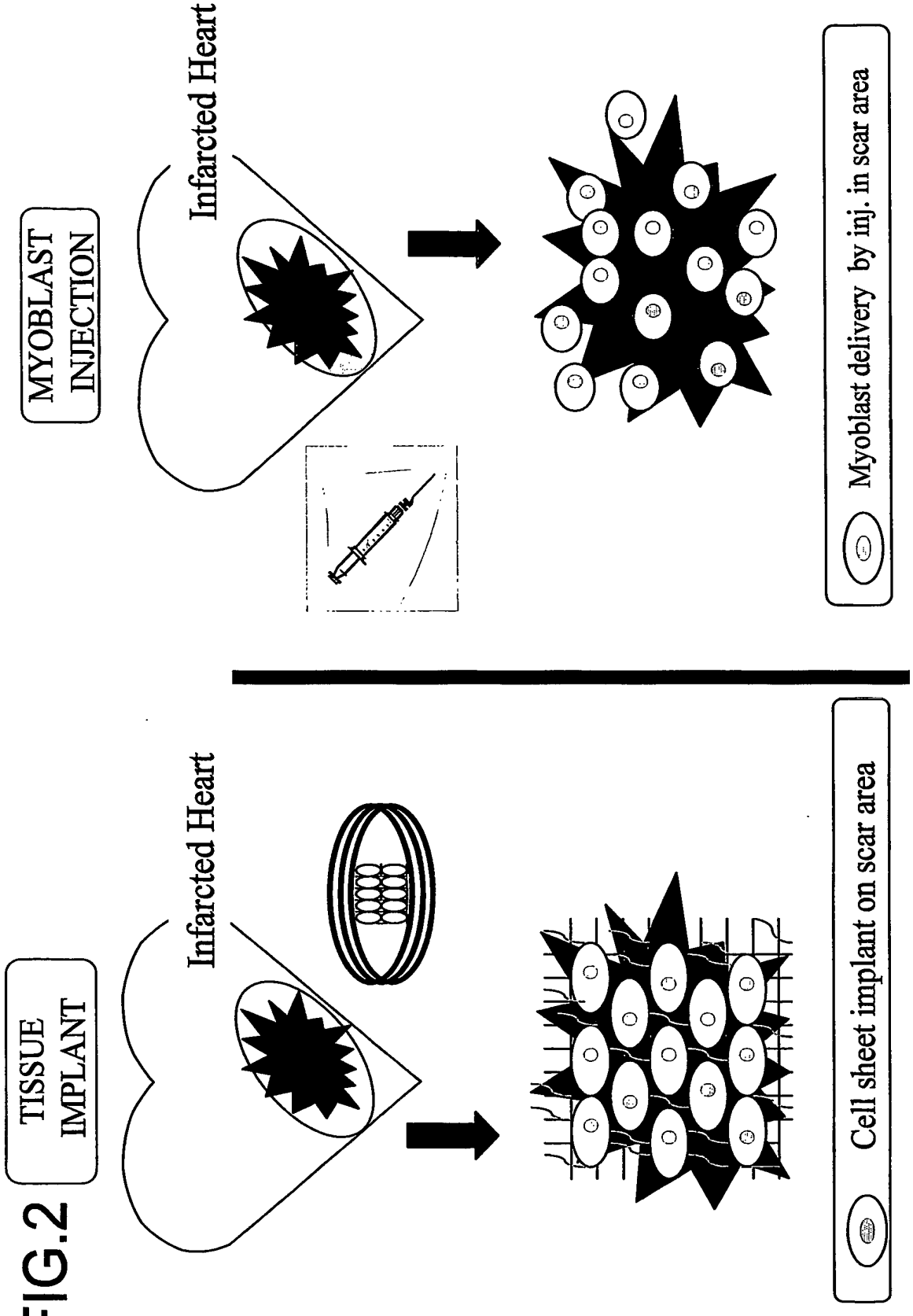

TISSUE IMPLANT
Infarcted Heart
Cell sheet implant on scar area
MYOBLAST INJECTION
Infarcted Heart
Myoblast delivery by inj. in scar area

FIG.3

Experimental Protocol

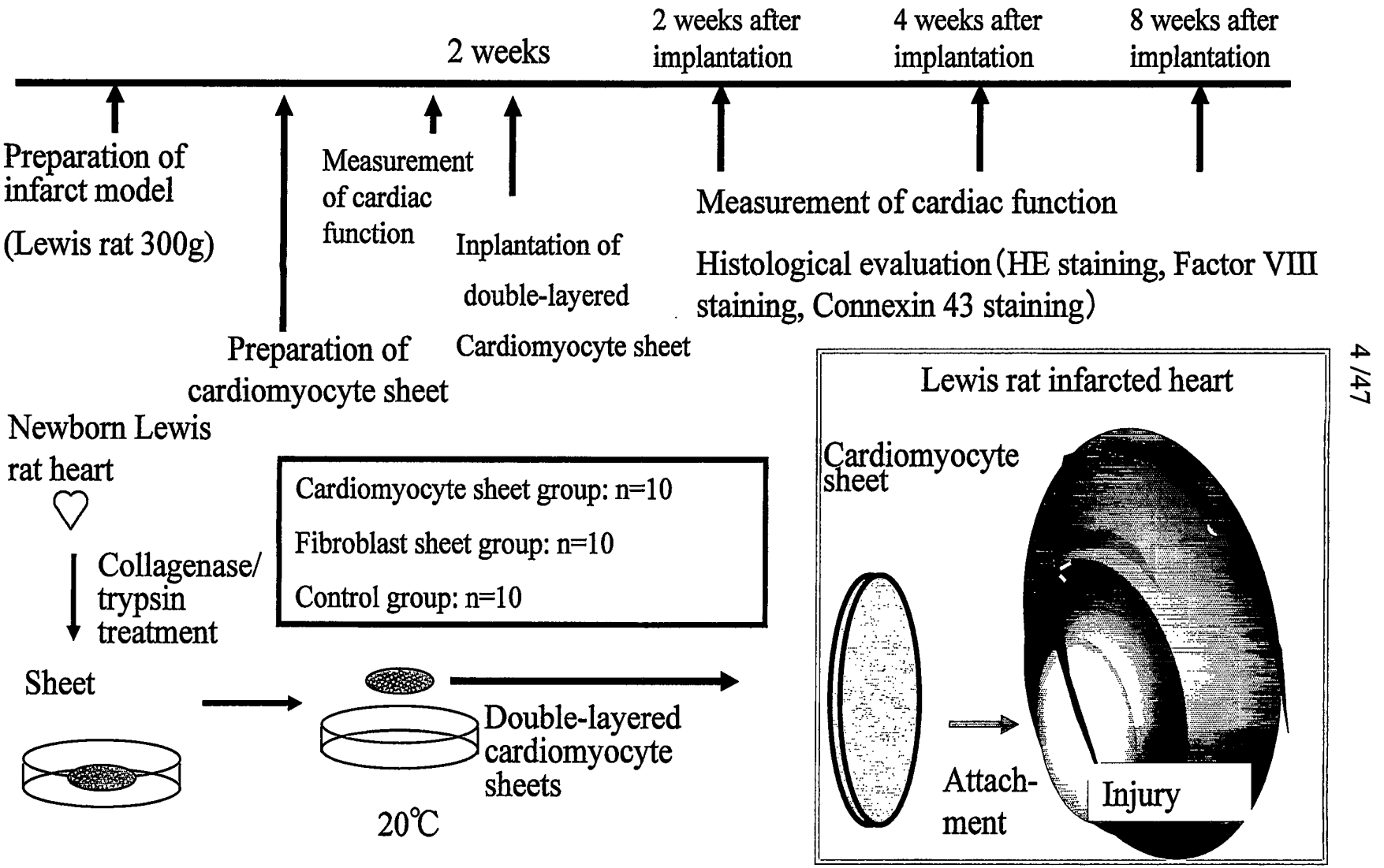


FIG.4 Regenerative therapy for cardiac muscle by cell transplantation

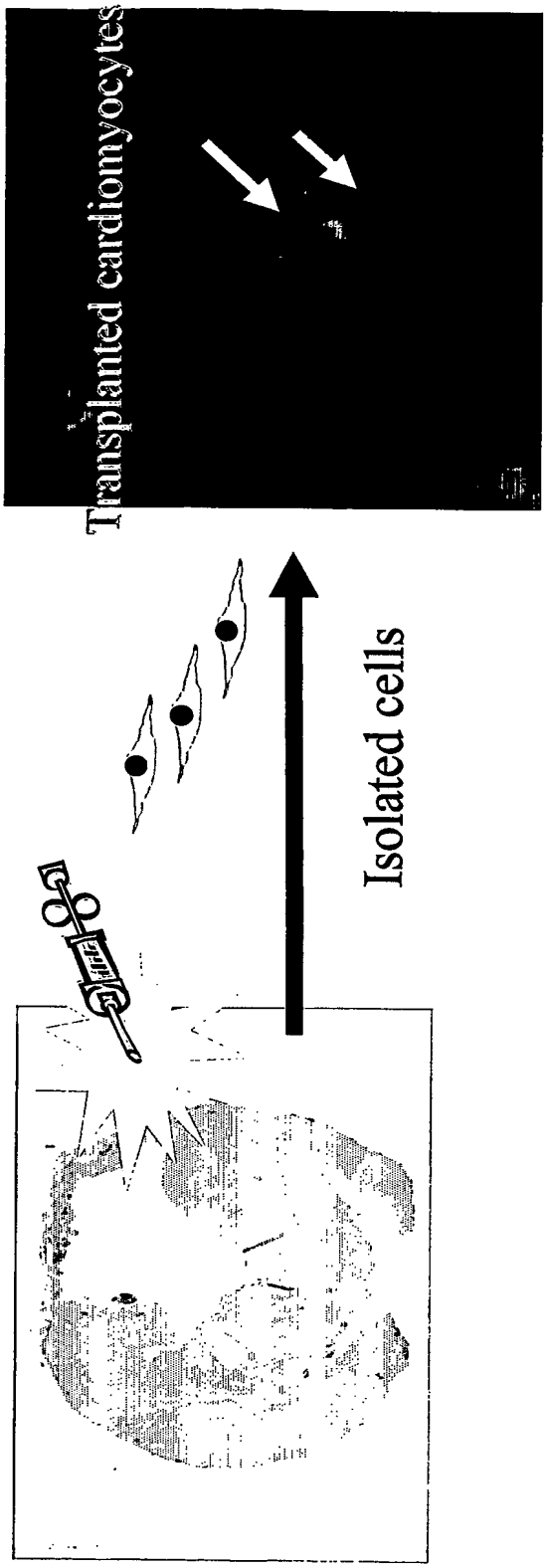


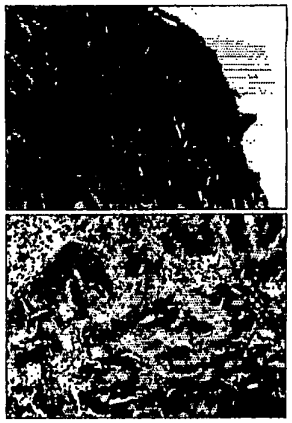
FIG.5 Problems with tissue transplantation

Cardiac muscle graft with scaffold

Alignment and cell-to-cell adhesion of transplanted cells within scaffold

Changes in scaffold in organism: elicitation of inflammation

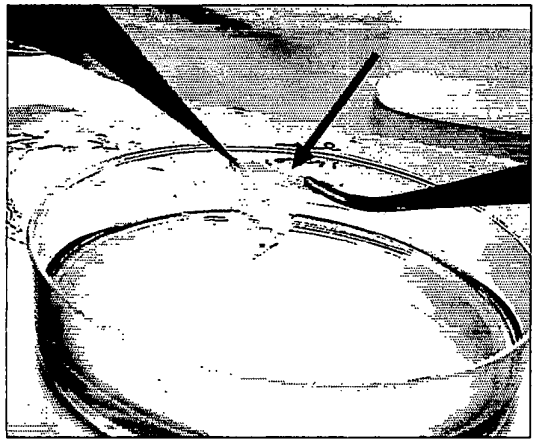
Acceptance of scaffold by recipient's heart



↓

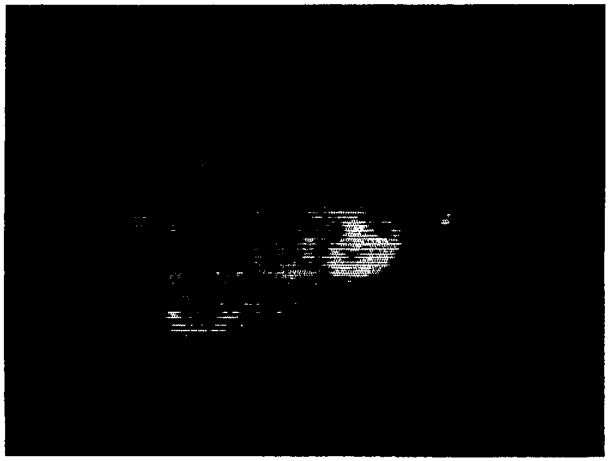
Development of high biocompatible cardiac muscle graft without scaffold

FIG.6 Implantation of cardiomyocyte sheet into infarcted heart

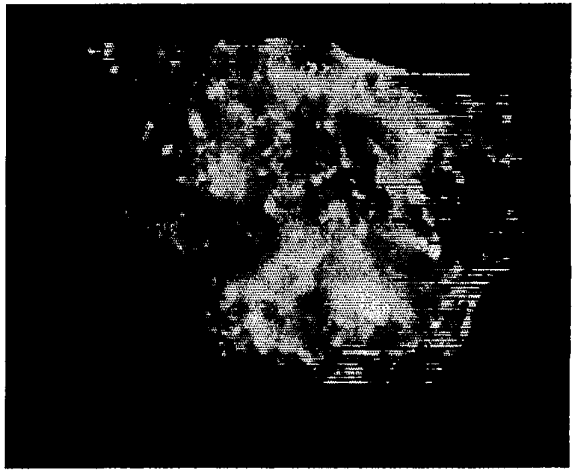


Cardiomyocyte sheet

Implantation into rat infarct model



In vitro



In vivo

Implantation of GFP rat newborn cardiomyocyte sheet

FIG.7

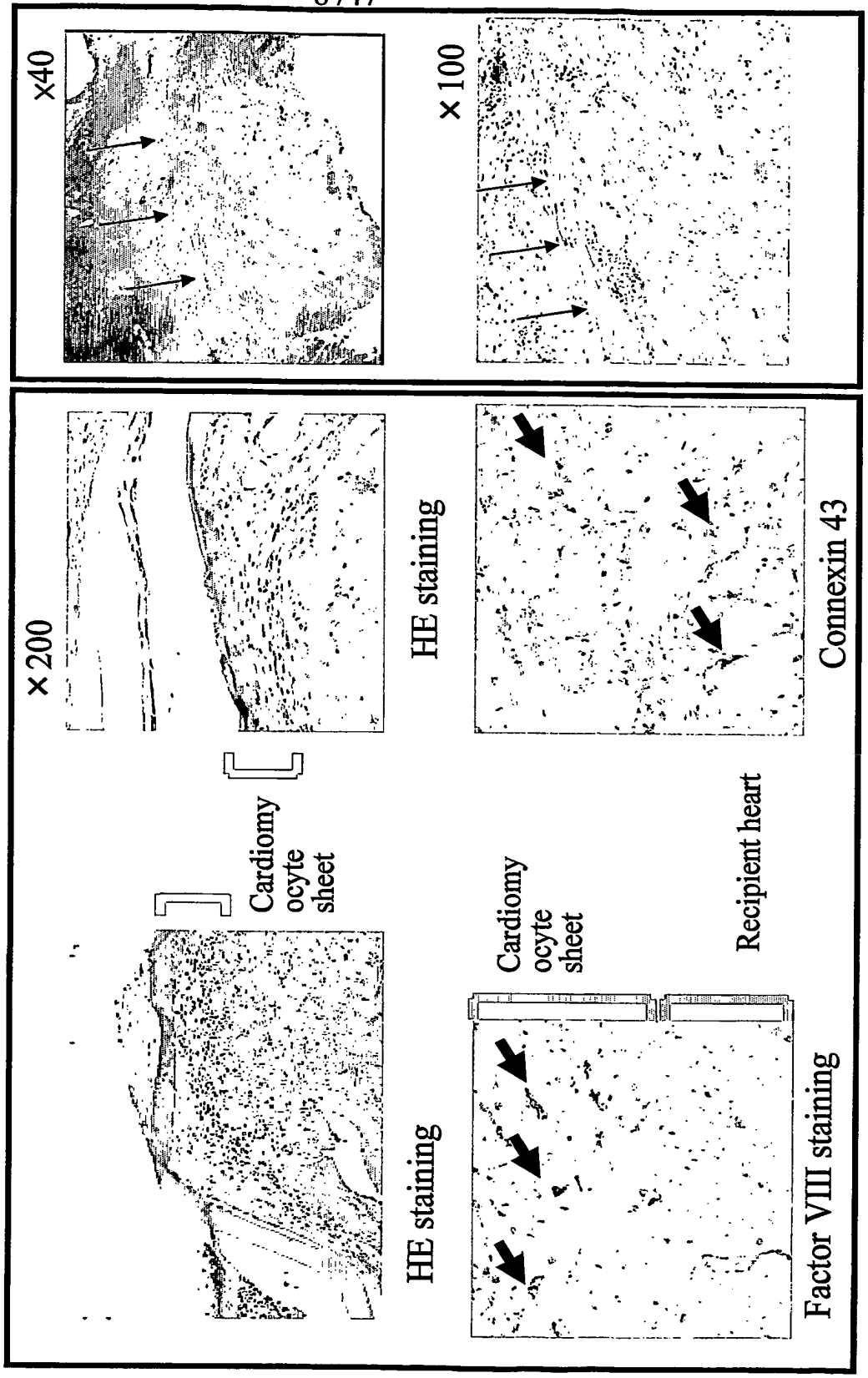
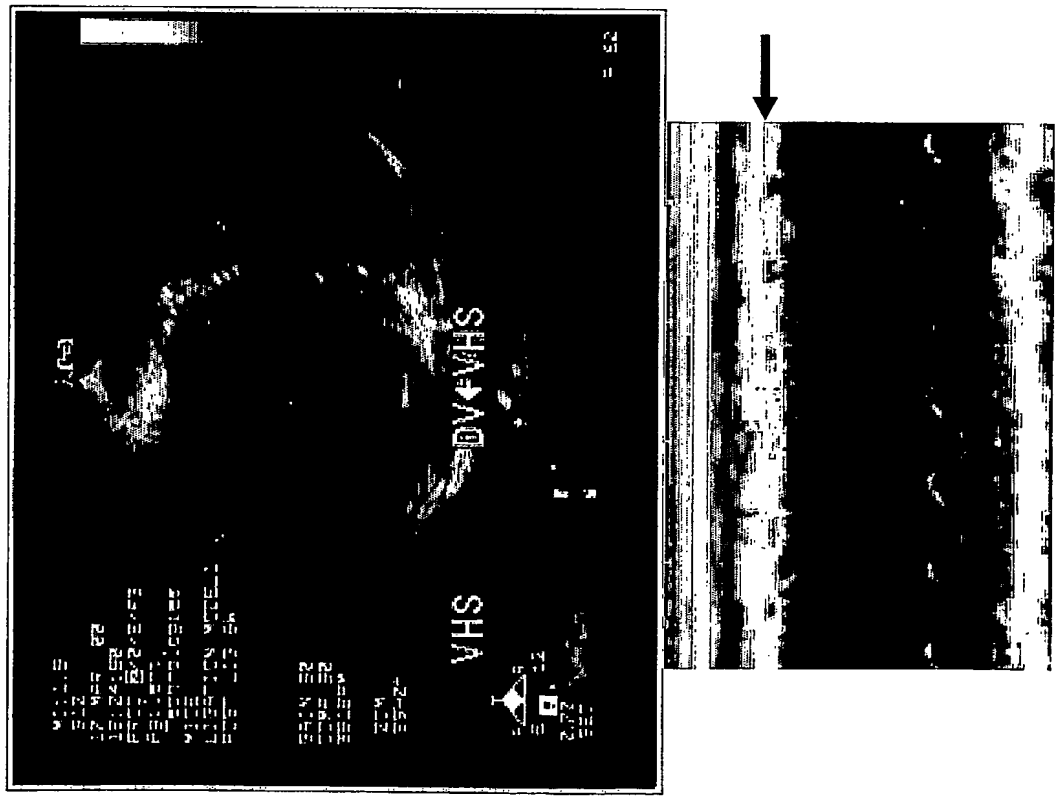

Tissue
2 weeks after implantation
8 weeks after implantation
× 200
HE staining
Cardiomyocyte sheet
HE staining
Factor VIII staining
Cardiomyocyte sheet
Recipient heart
Connexin 43
×40
× 100

FIG.8 Evaluation of Cardiac function - 1

Control



Implantation of prosthetic tissue

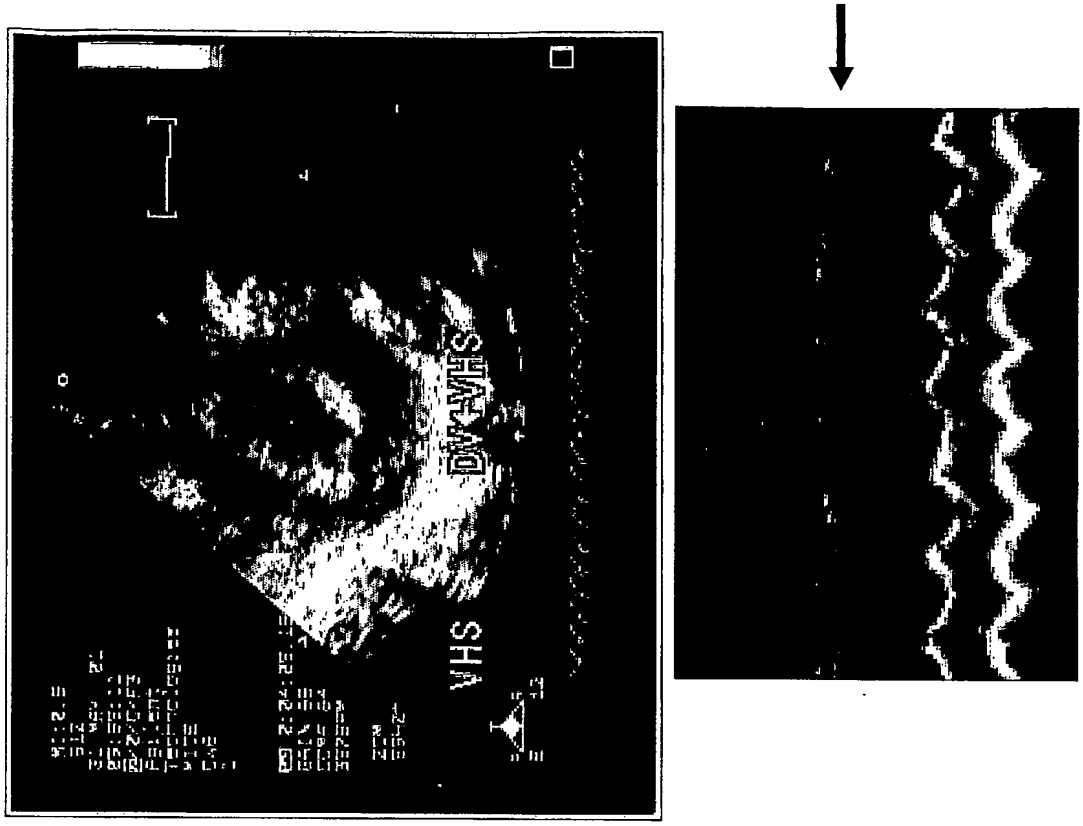


FIG.9

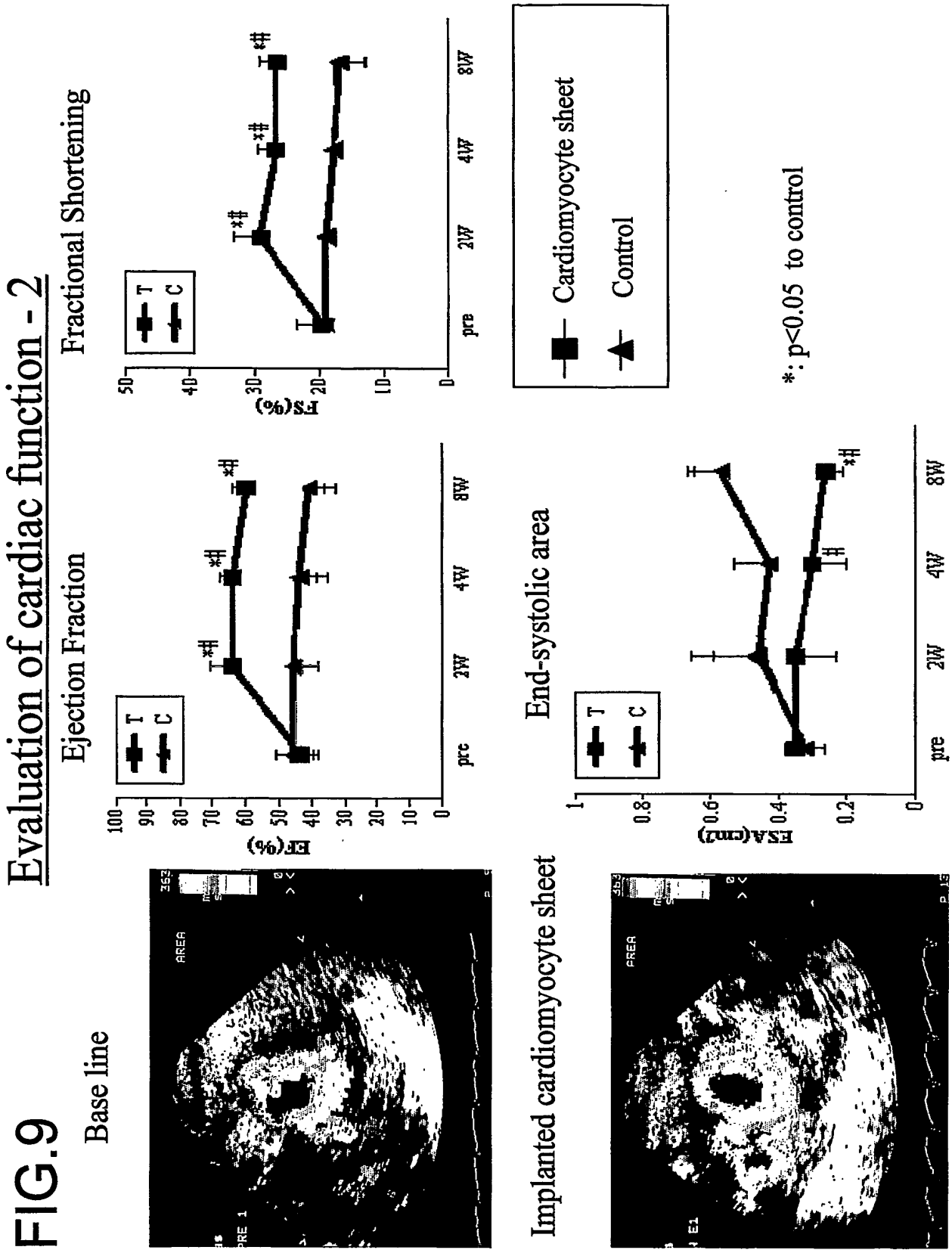
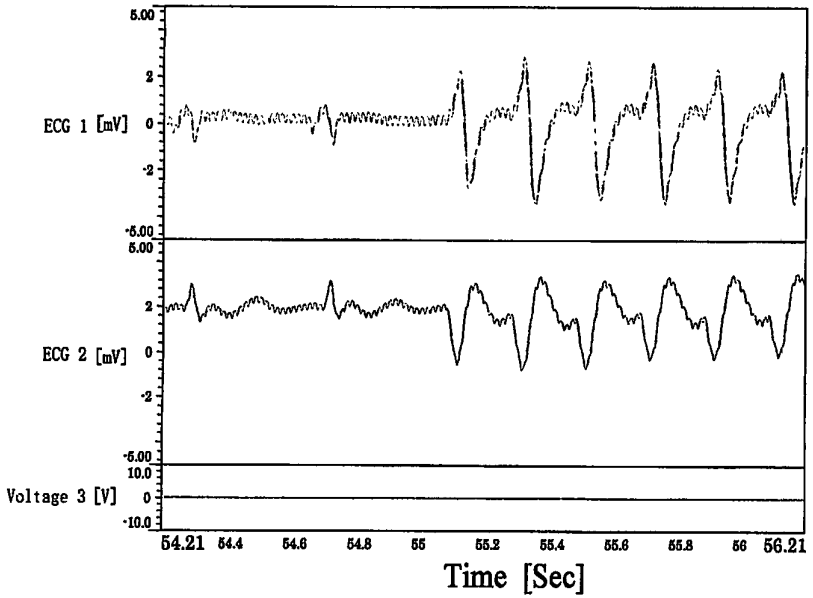
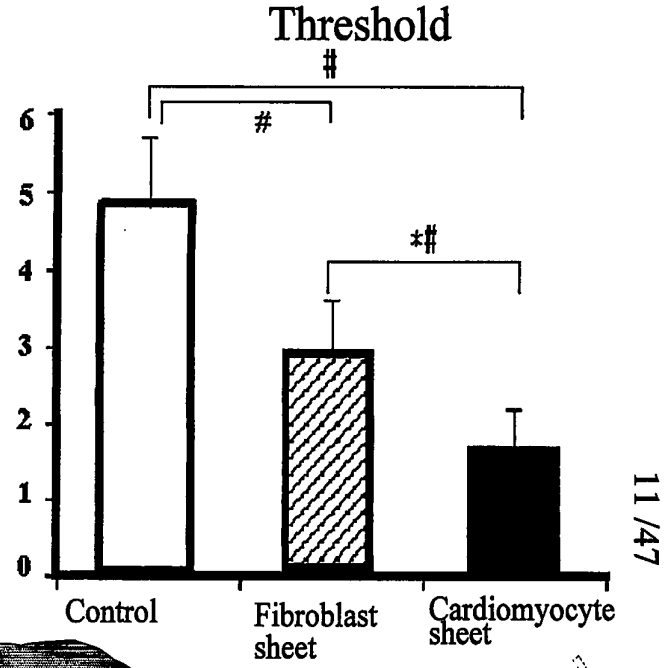
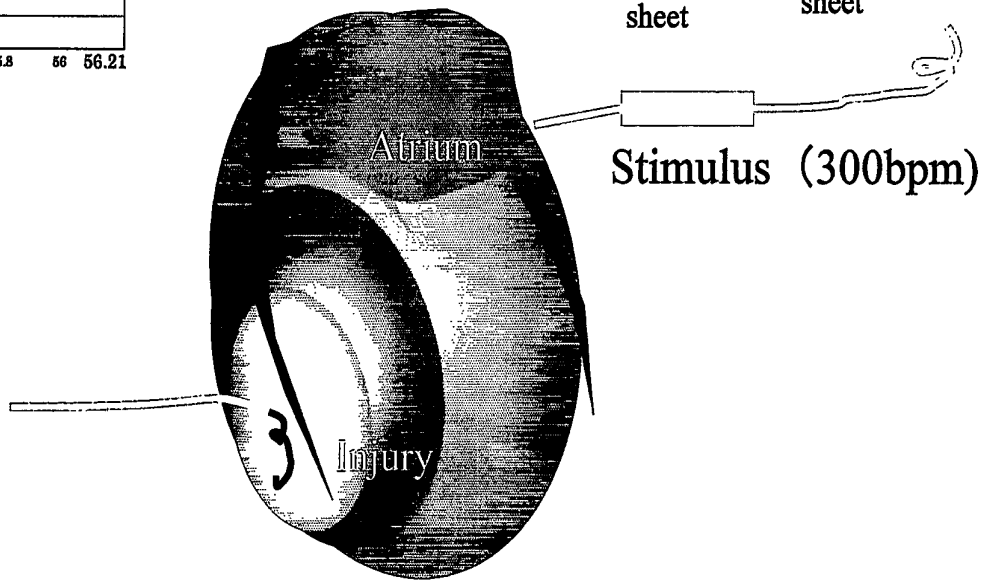

Evaluation of cardiac function - 2
Base line
Ejection Fraction
EF(%)
Fractional Shortening
FS(%)
Implanted cardiomyocyte sheet
End-systolic area
ESA(cm2)
T
C
pre
2W
4W
8W
Cardiomyocyte sheet
Control
*: p<0.05 to control

FIG.10

Electrophysiological Evaluation

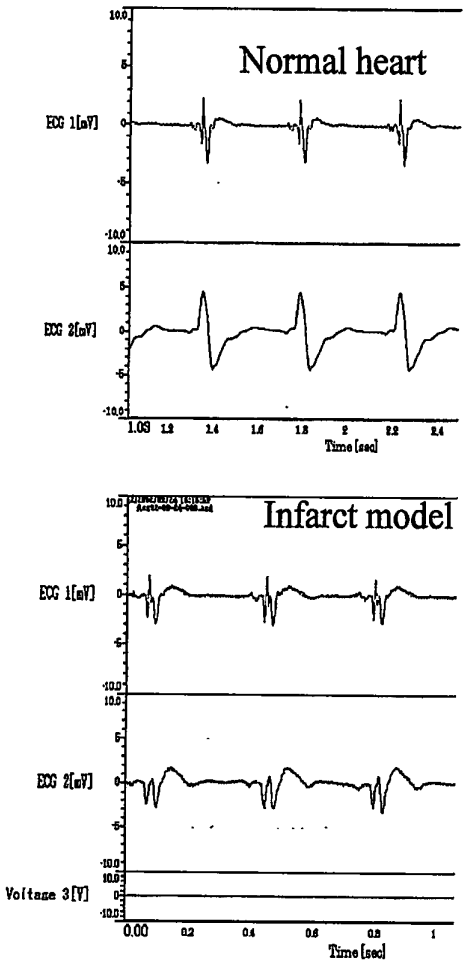






Buried electrode at sheet implanted site

FIG.11



ECG 1:ECG (Surface)

ECG 2: Normal heart (anterior wall)

Ligation model (injured)

Prosthetic tissue implanted

(prosthetic tissue injured)

Electrophysiological Evaluation

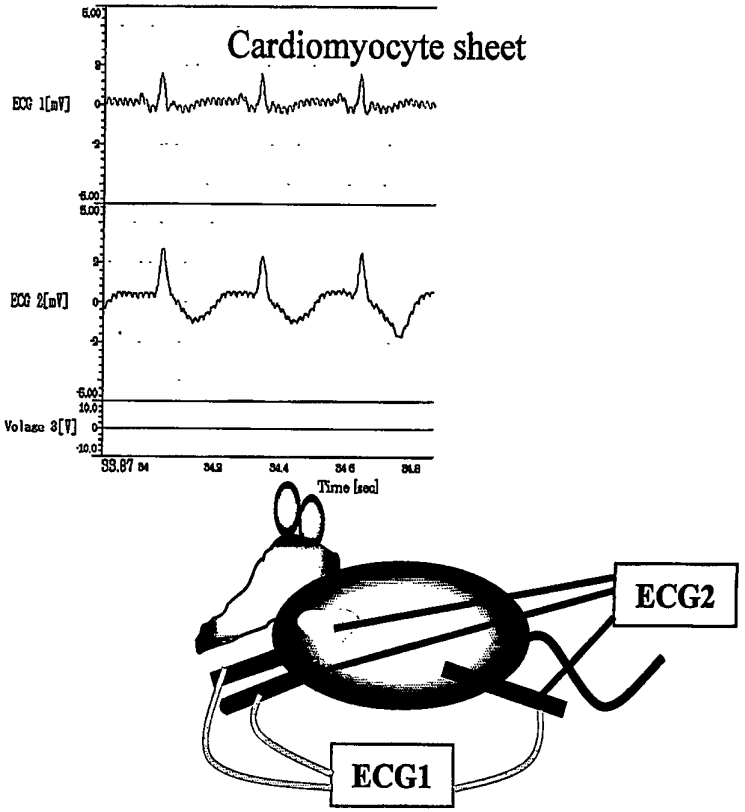


FIG.12

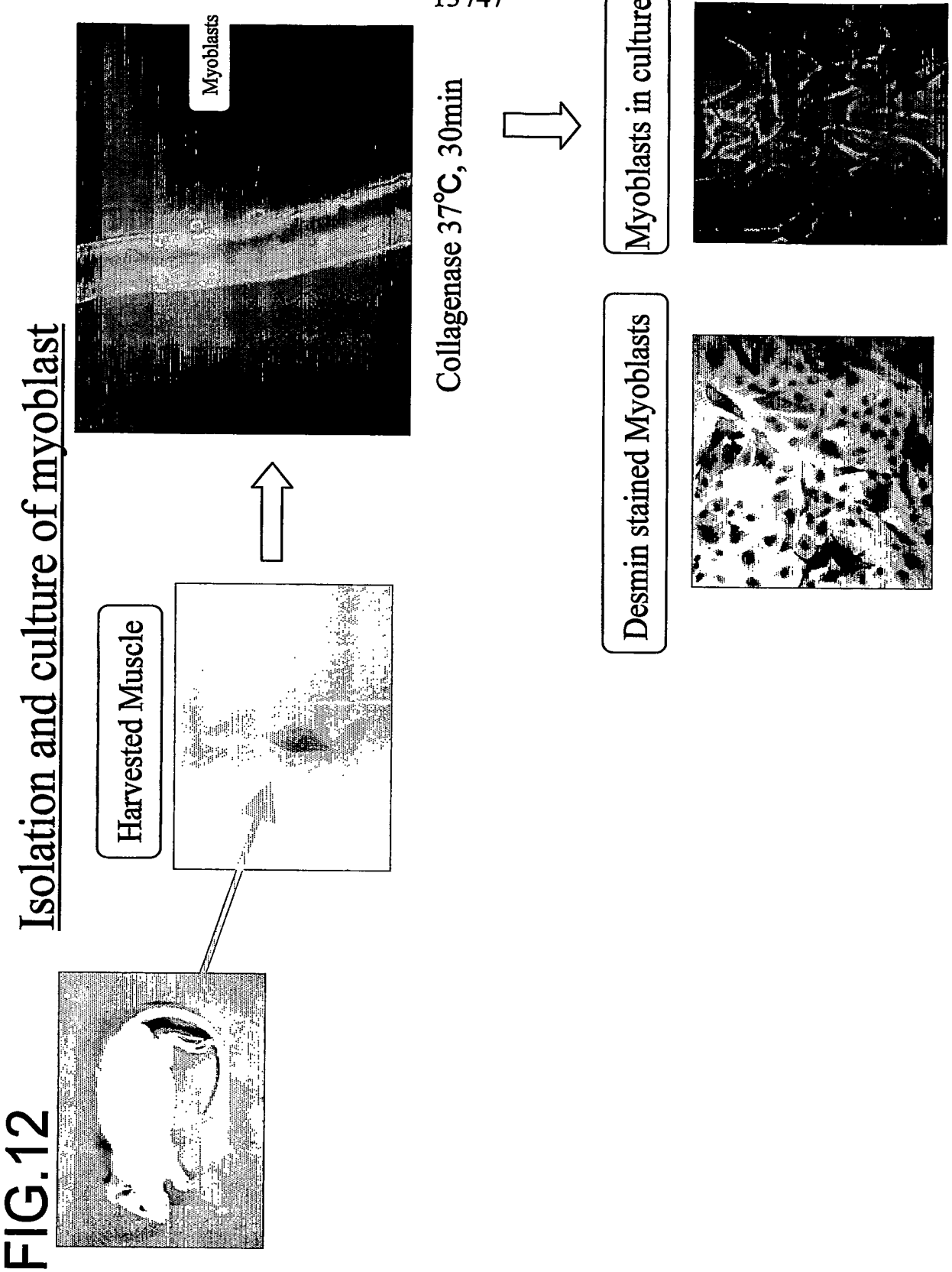

Isolation and culture of myoblast
Harvested Muscle
Myoblasts
Collagenase 37°C, 30min
Myoblasts in culture
Desmin stained Myoblasts

FIG.13

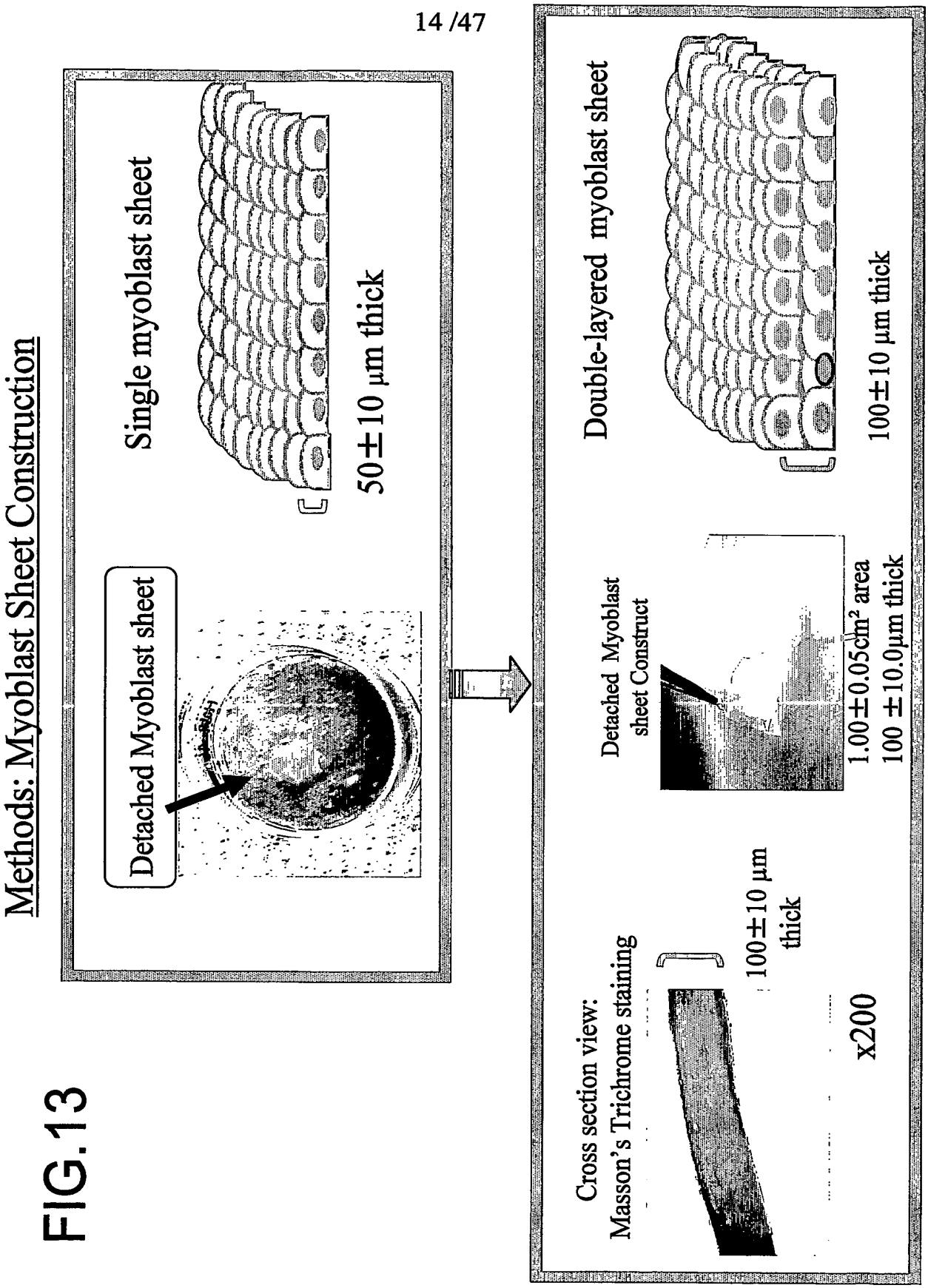

Methods: Myoblast Sheet Construction
Single myoblast sheet
Detached Myoblast sheet
50±10 μm thick
Double-layered myoblast sheet
100±10 μm thick
Detached Myoblast sheet Construct
1.00±0.05cm² area
100 ±10.0μm thick
Cross section view:
Masson's Trichrome staining
100±10 μm thick
x200

FIG.14

Experimental Protocol

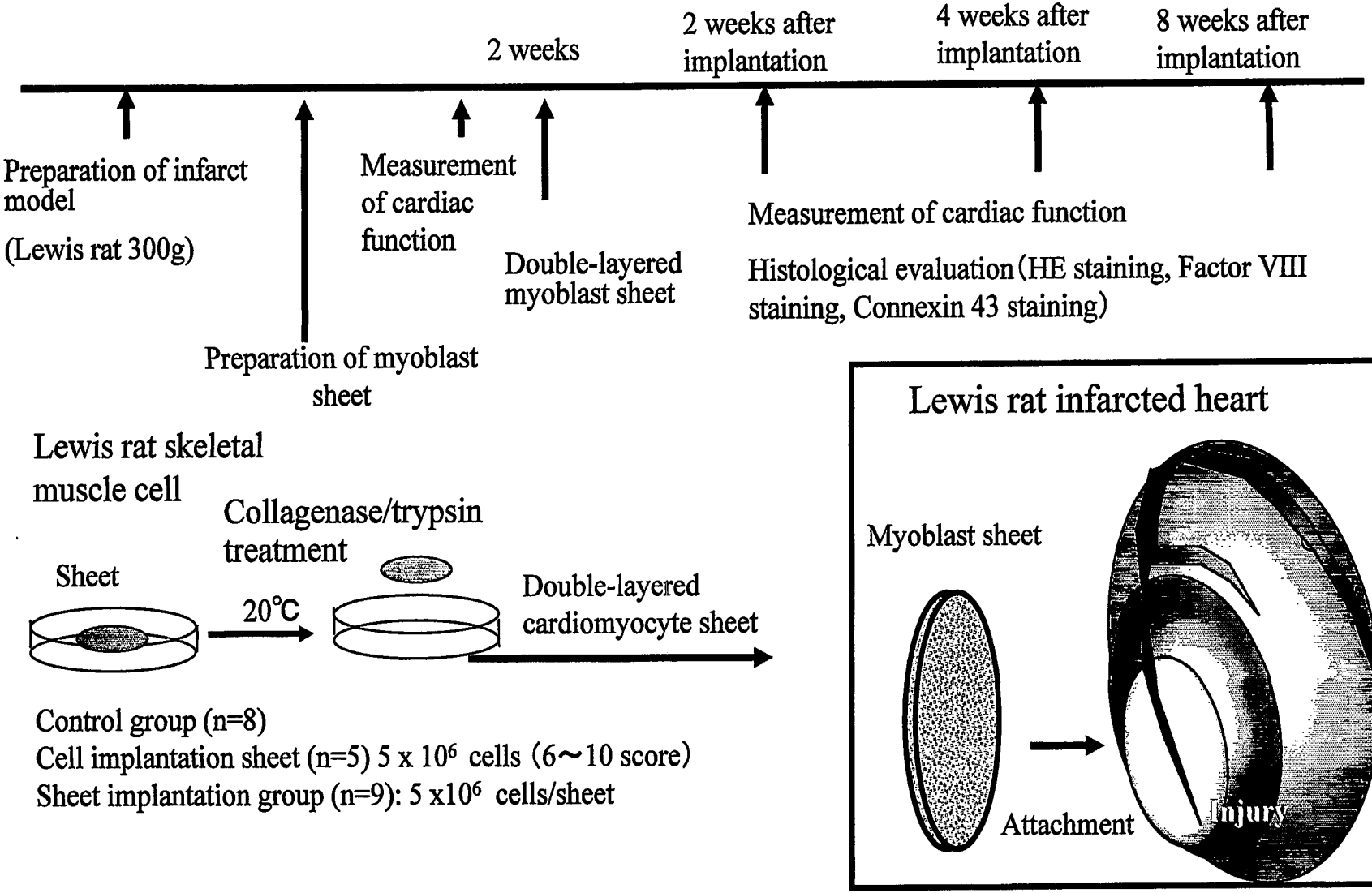


FIG.15

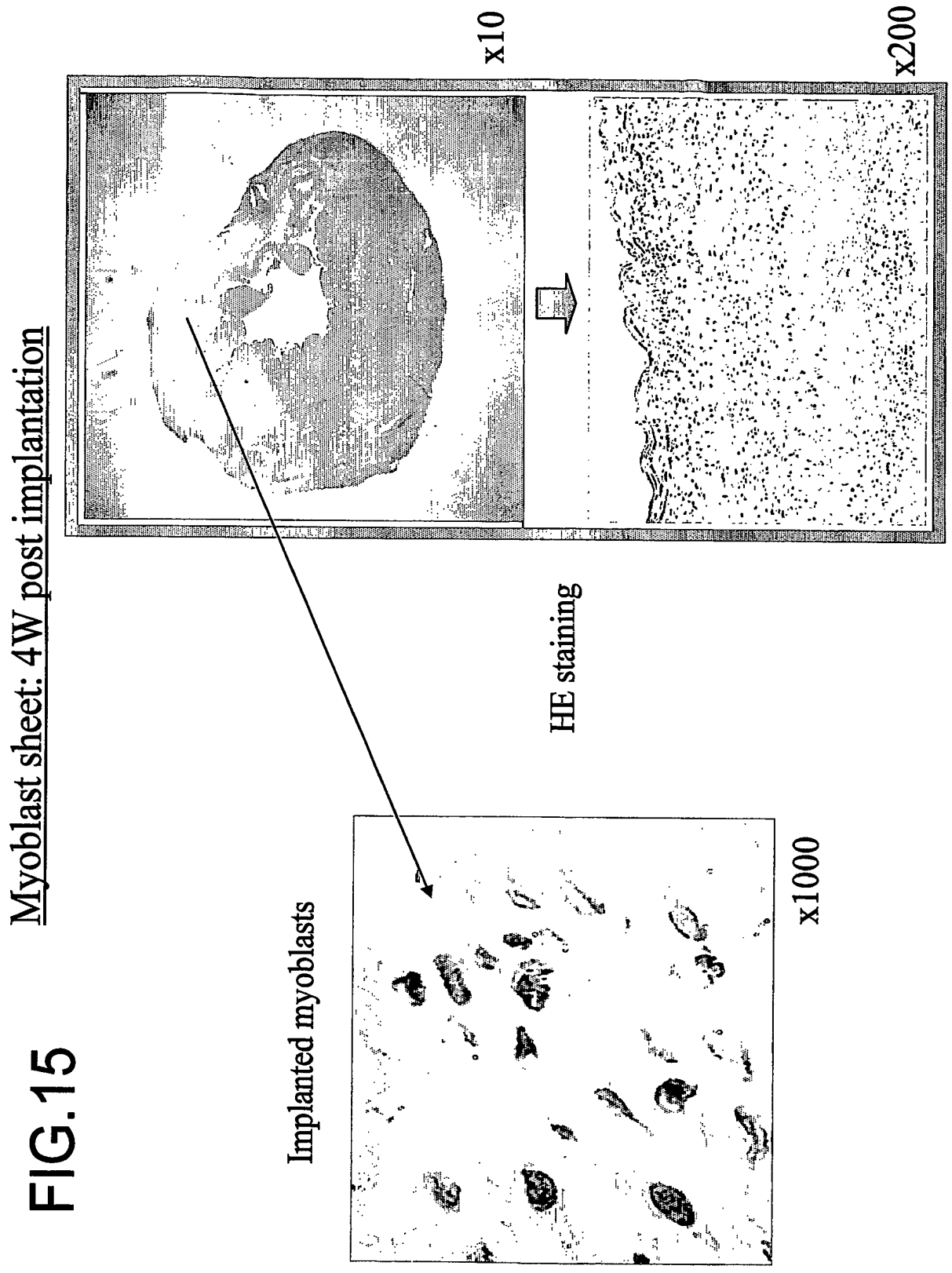

Myoblast sheet: 4W post implantation
x10
x200
HE staining
Implanted myoblasts
x1000

FIG.16 Myoblast sheet Implantation procedure

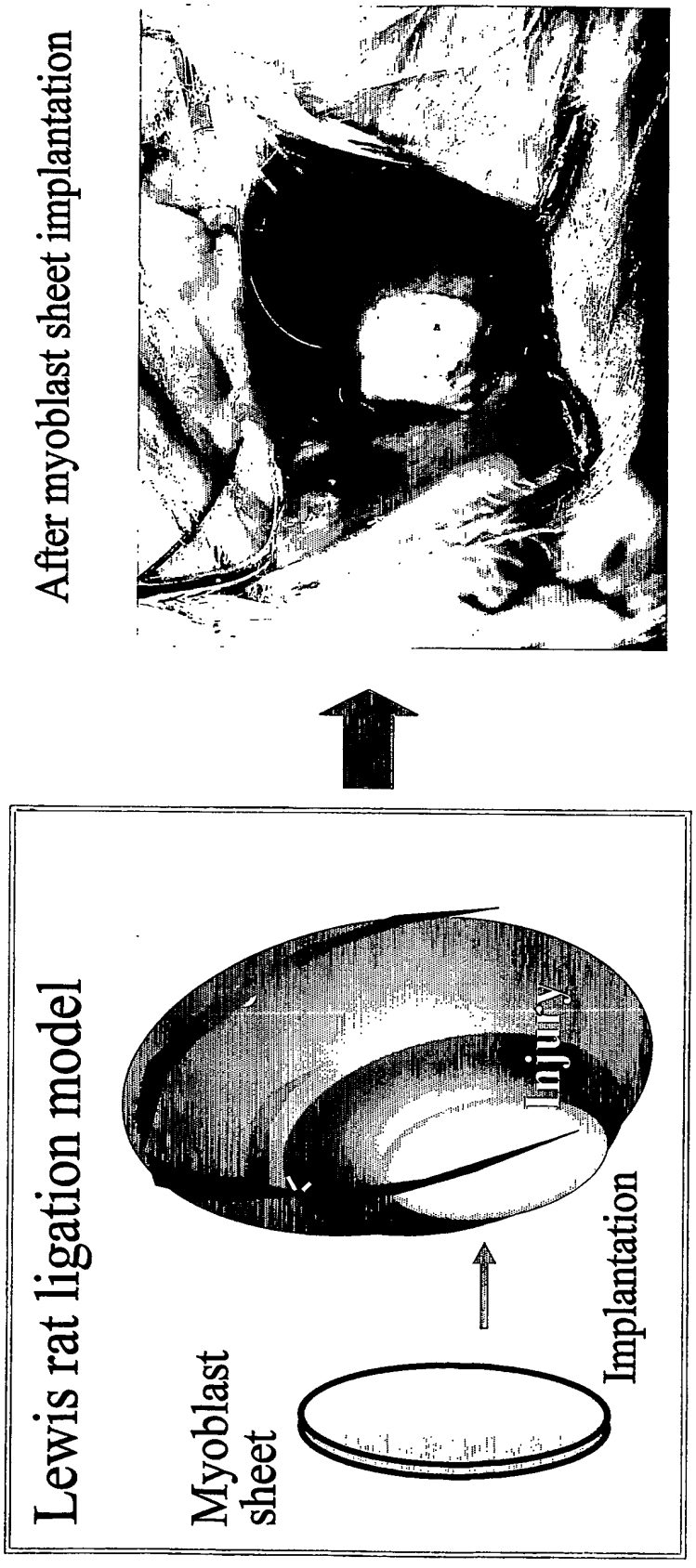


FIG.17 HistologyMasson's Trichrome staining

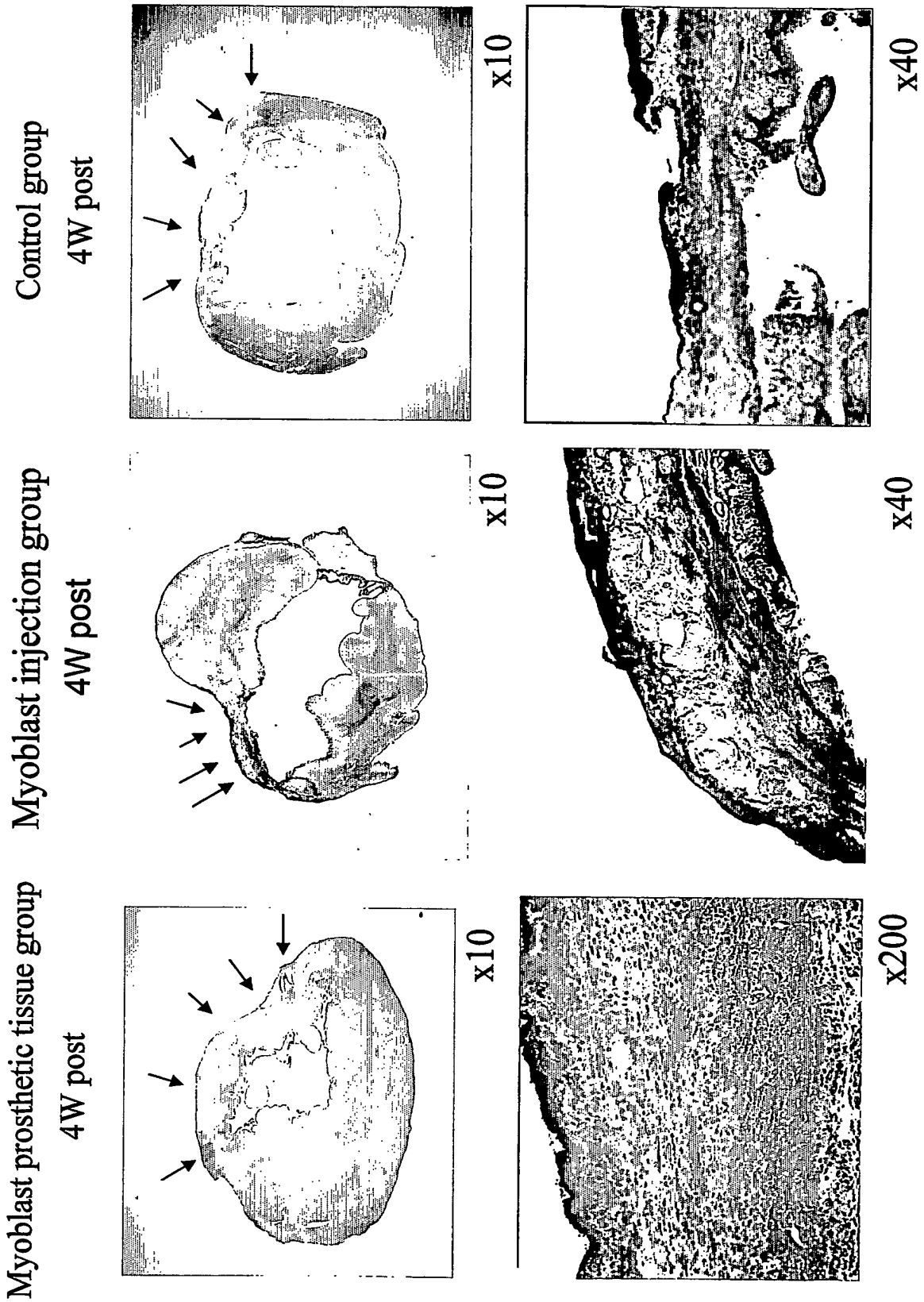


FIG.18

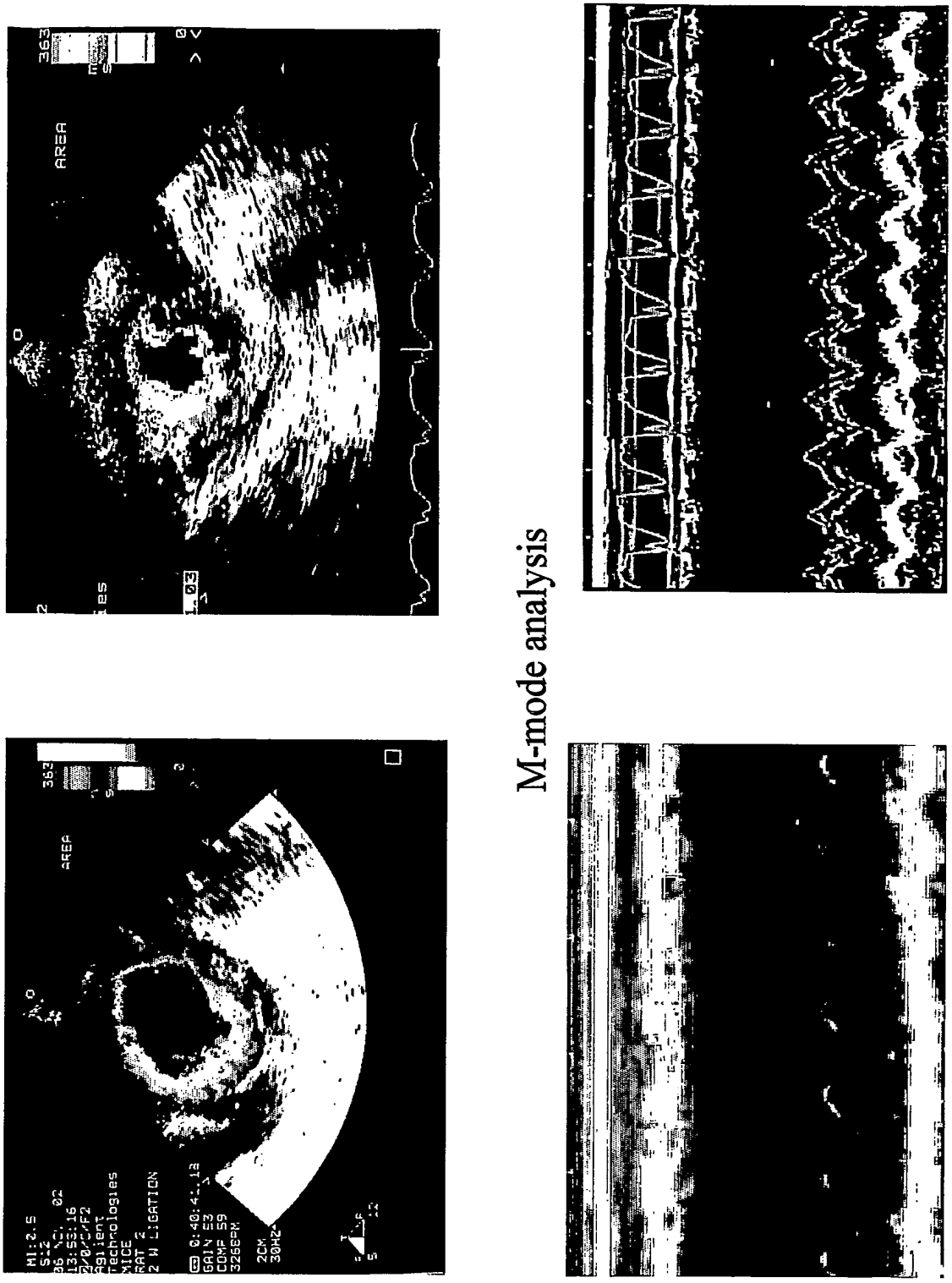
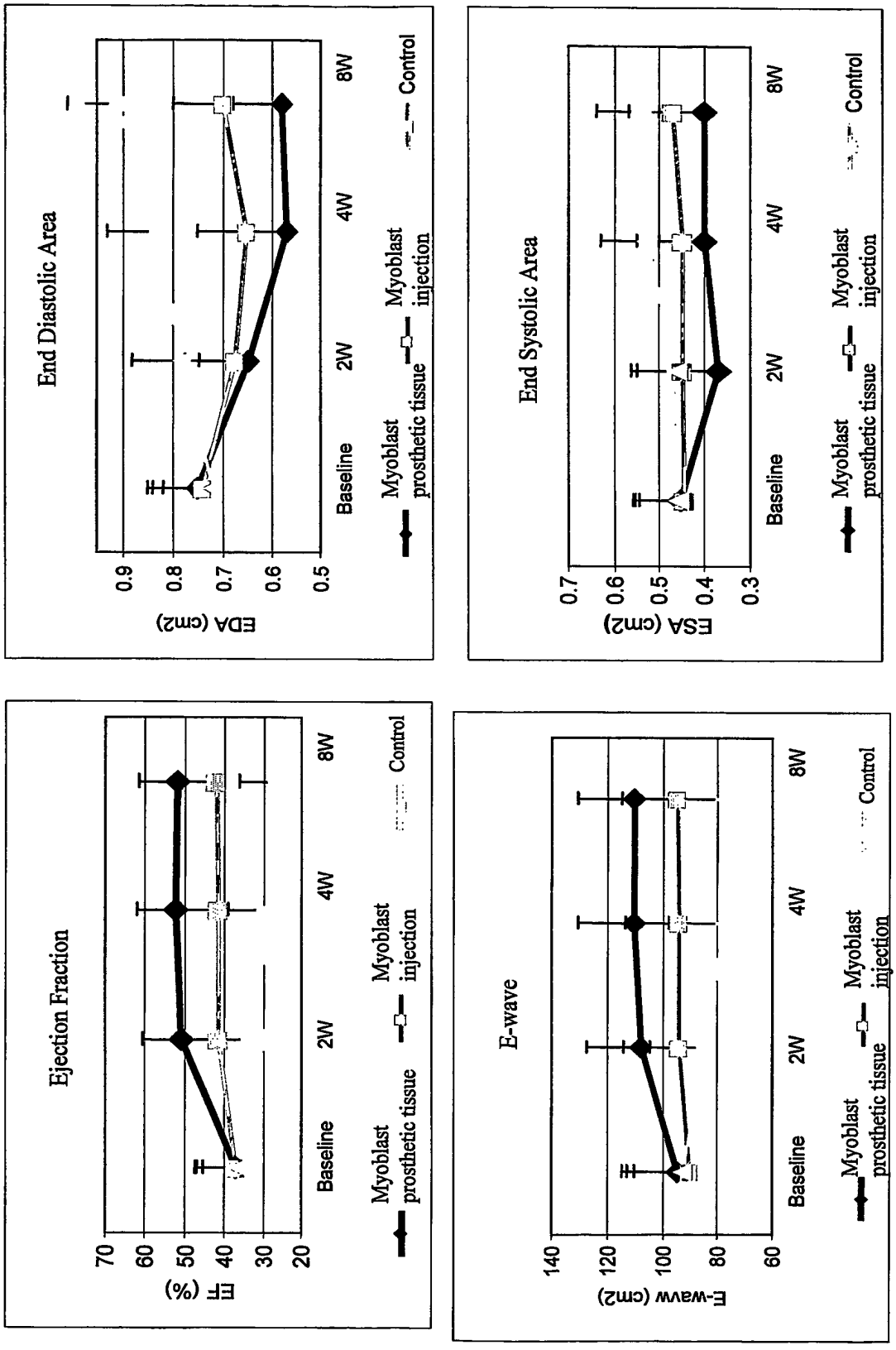


FIG.19



#P< 0.05 for control; *P< 0.05 to for injection needle group

FIG.20

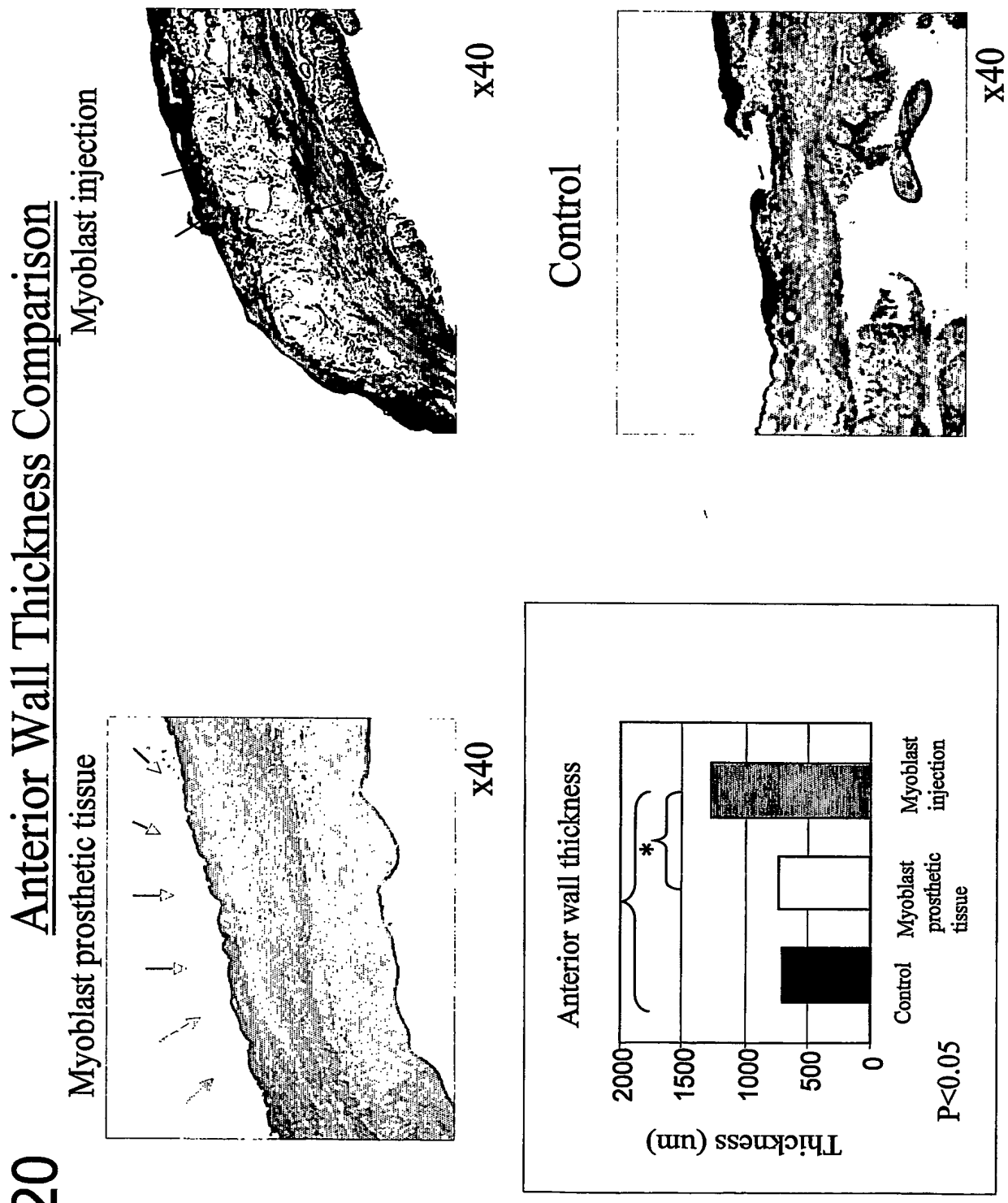

Anterior Wall Thickness Comparison
Myoblast prosthetic tissue
x40
Myoblast injection
x40
Control
x40
Anterior wall thickness
Thickness (um)
2000
1500
1000
500
0
*
Control
Myoblast prosthetic tissue
Myoblast injection
P<0.05

FIG.21

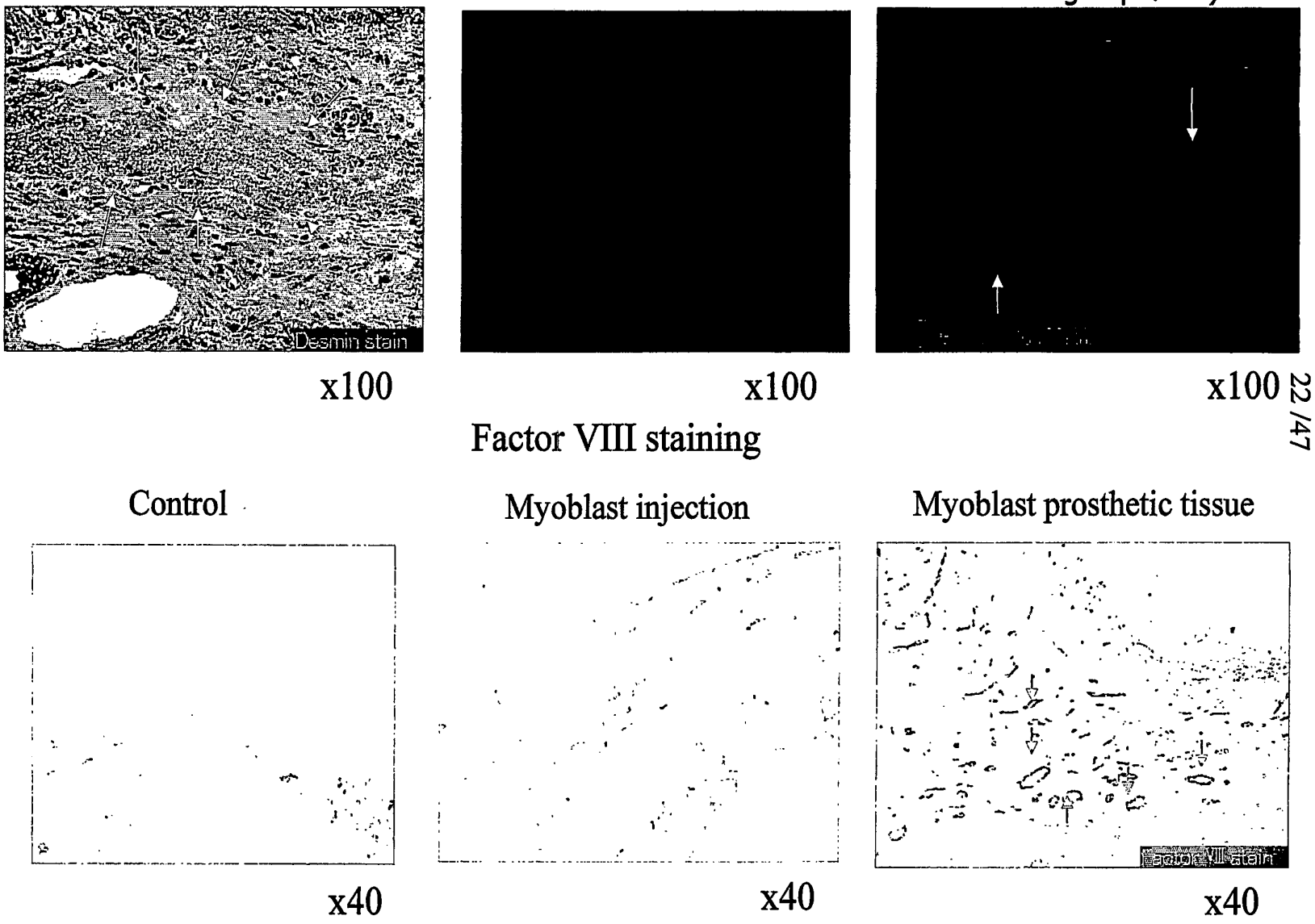

Myoblast sheet:
Desmin Staining
Control group (GFP)
Myoblast prosthetic tissue group (GFP)
Desmin stain
x100
x100
x100
Factor VIII staining
Control
Myoblast injection
Myoblast prosthetic tissue
x40
x40
x40

FIG.22A

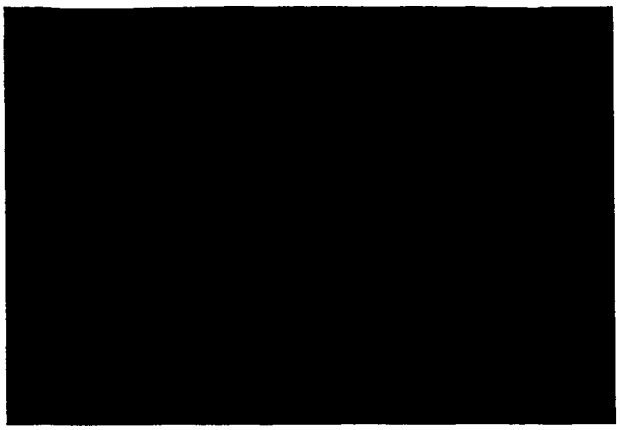


FIG.22B

FIG.22C

FIG.22D

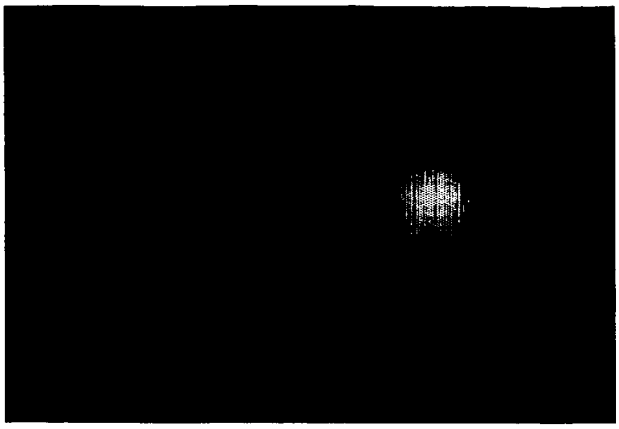


FIG.22E

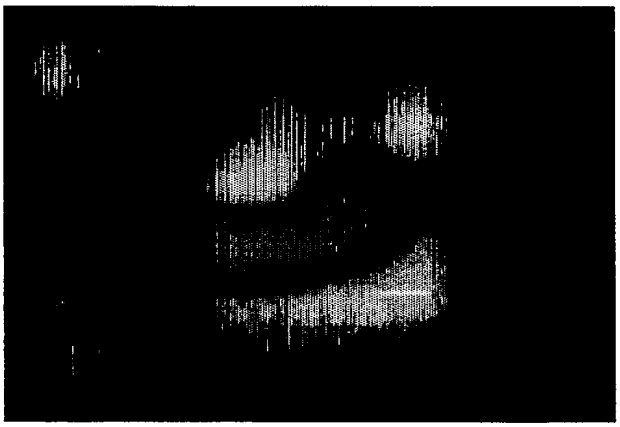


FIG.22F

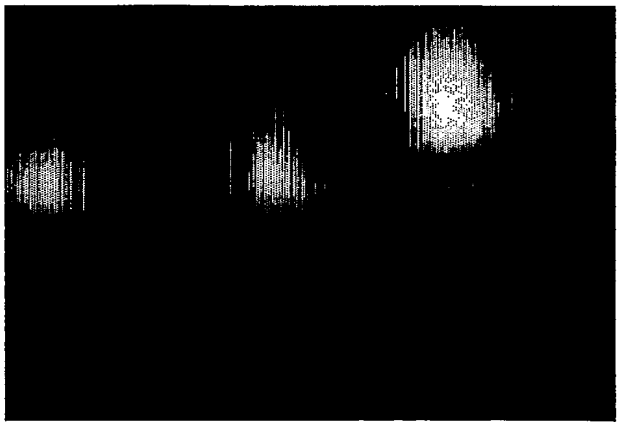


FIG.23A

FIG.23B

FIG.23C

FIG.24A

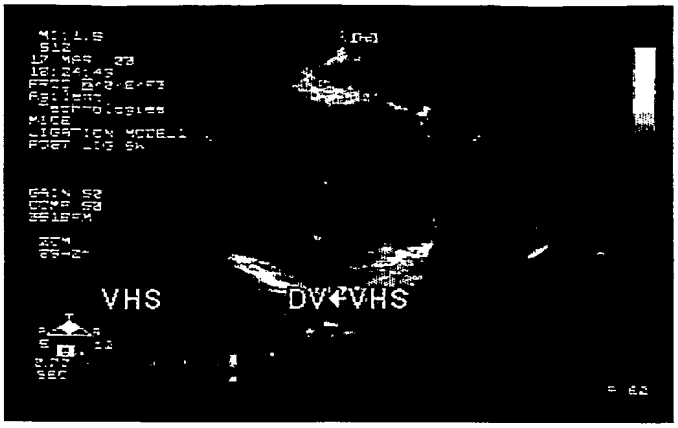


FIG.24B

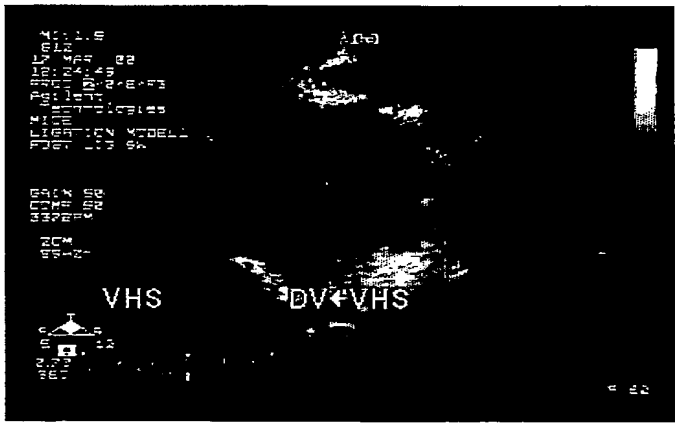


FIG.24C

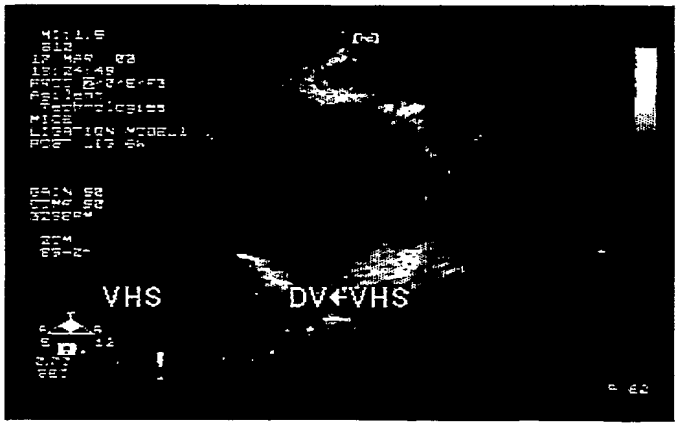


FIG.25A

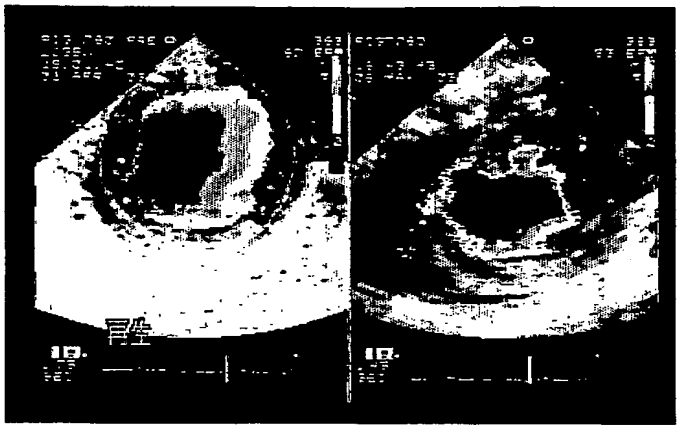


FIG.25B

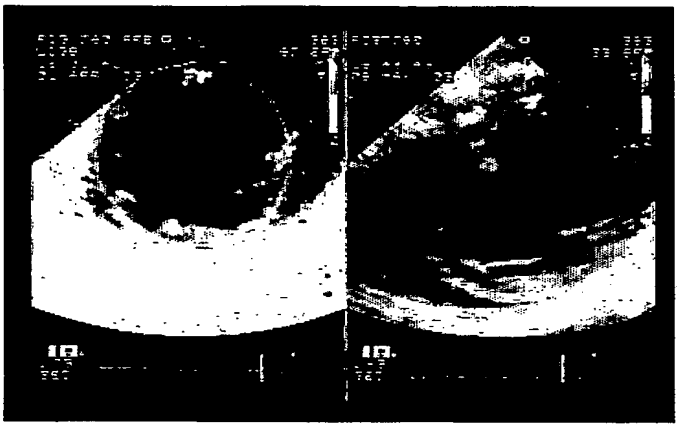


FIG.25C

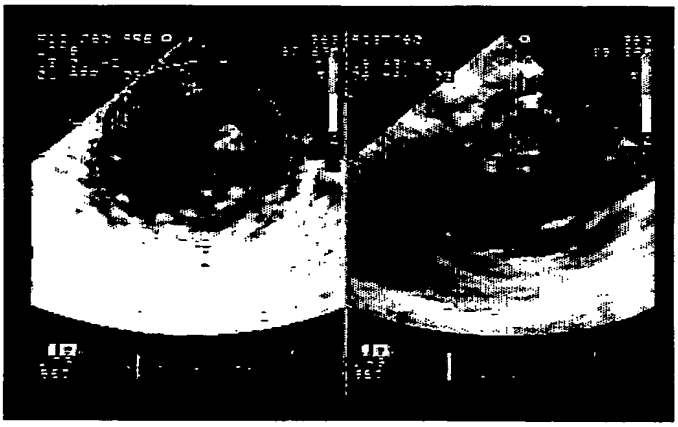


FIG.26A

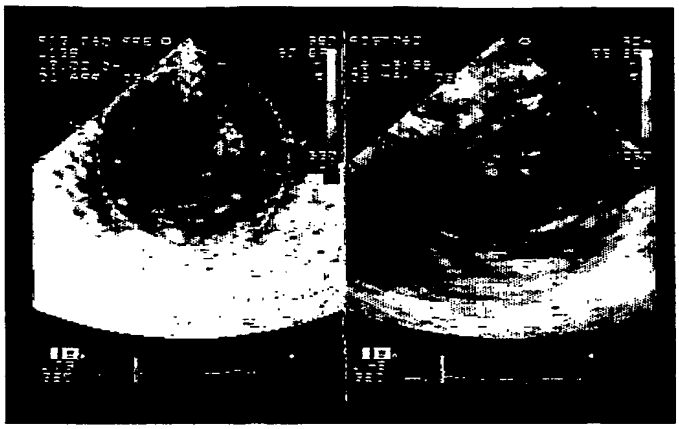


FIG.26B

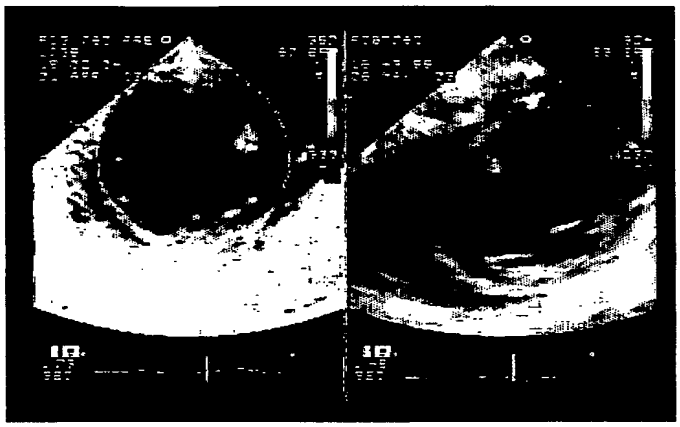


FIG.26C

FIG.27A

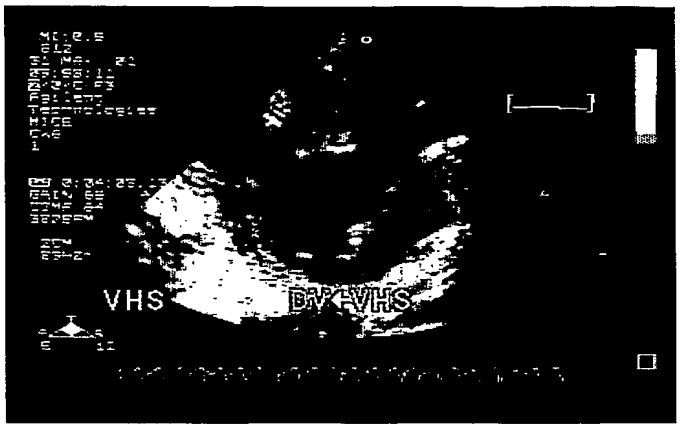


FIG.27B

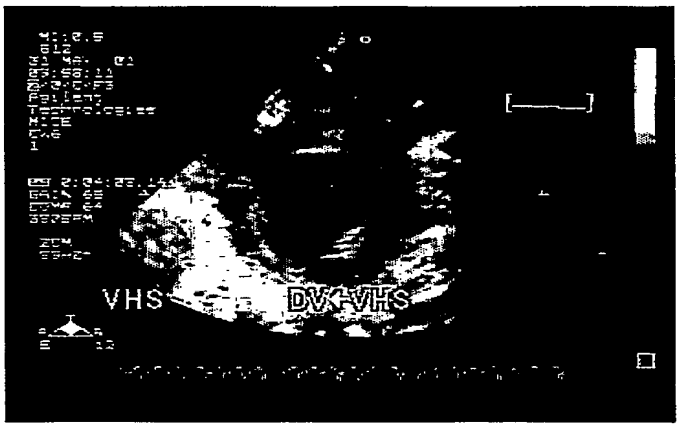


FIG.27C

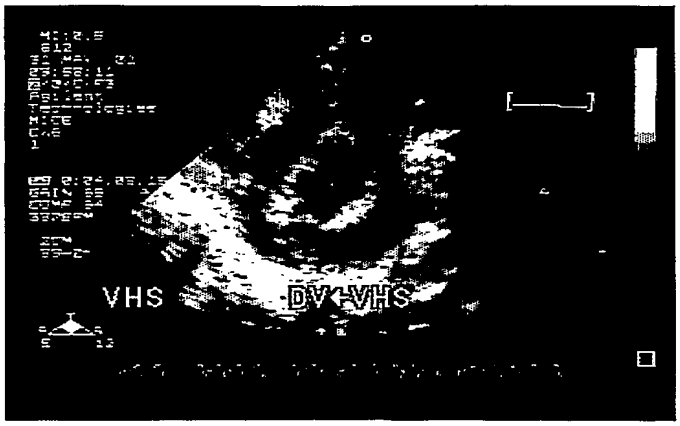


FIG.28

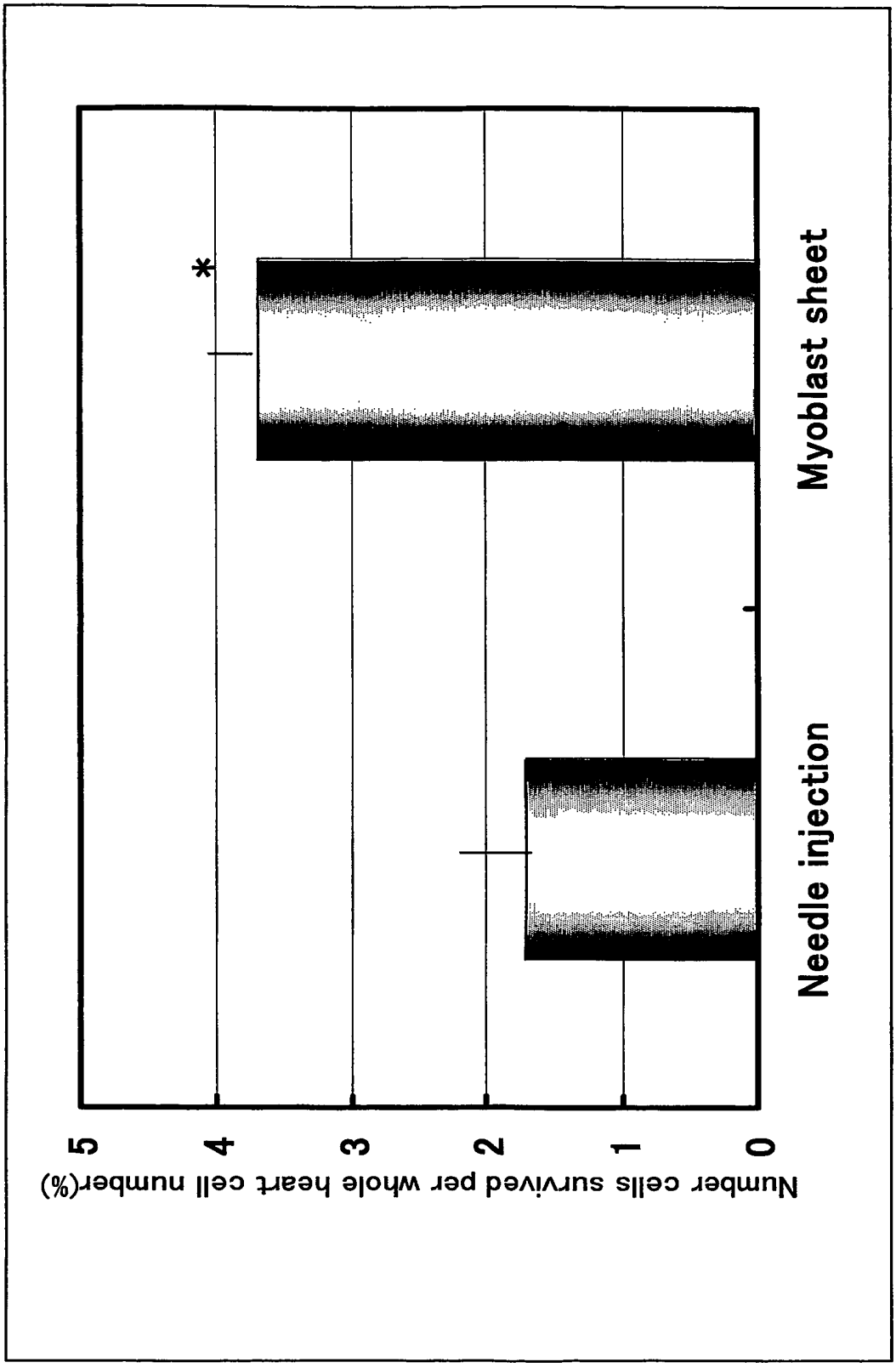

Number cells survived per whole heart cell number(%)
0
1
2
3
4
5
*
Myoblast sheet
Needle injection

FIG.29

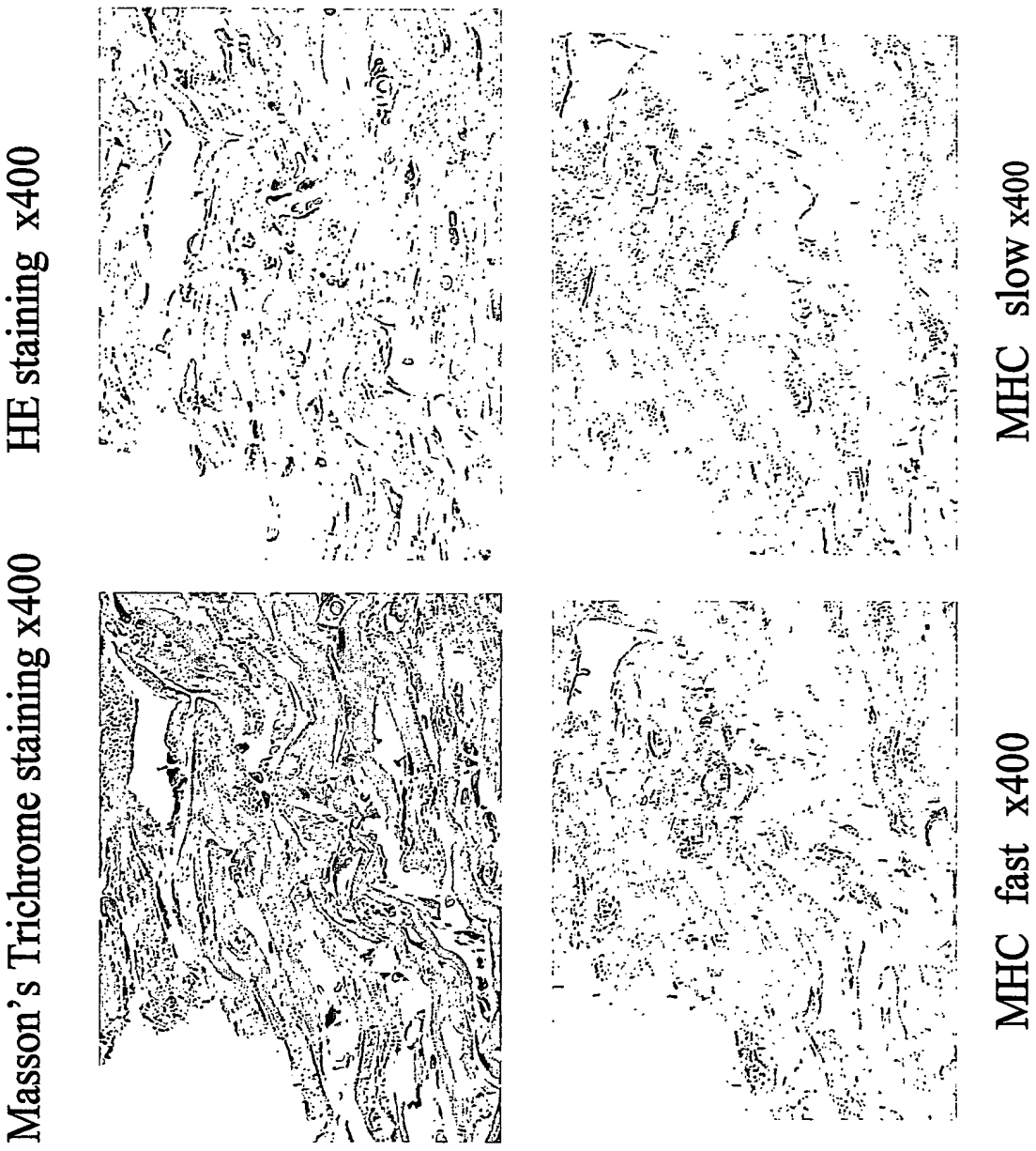
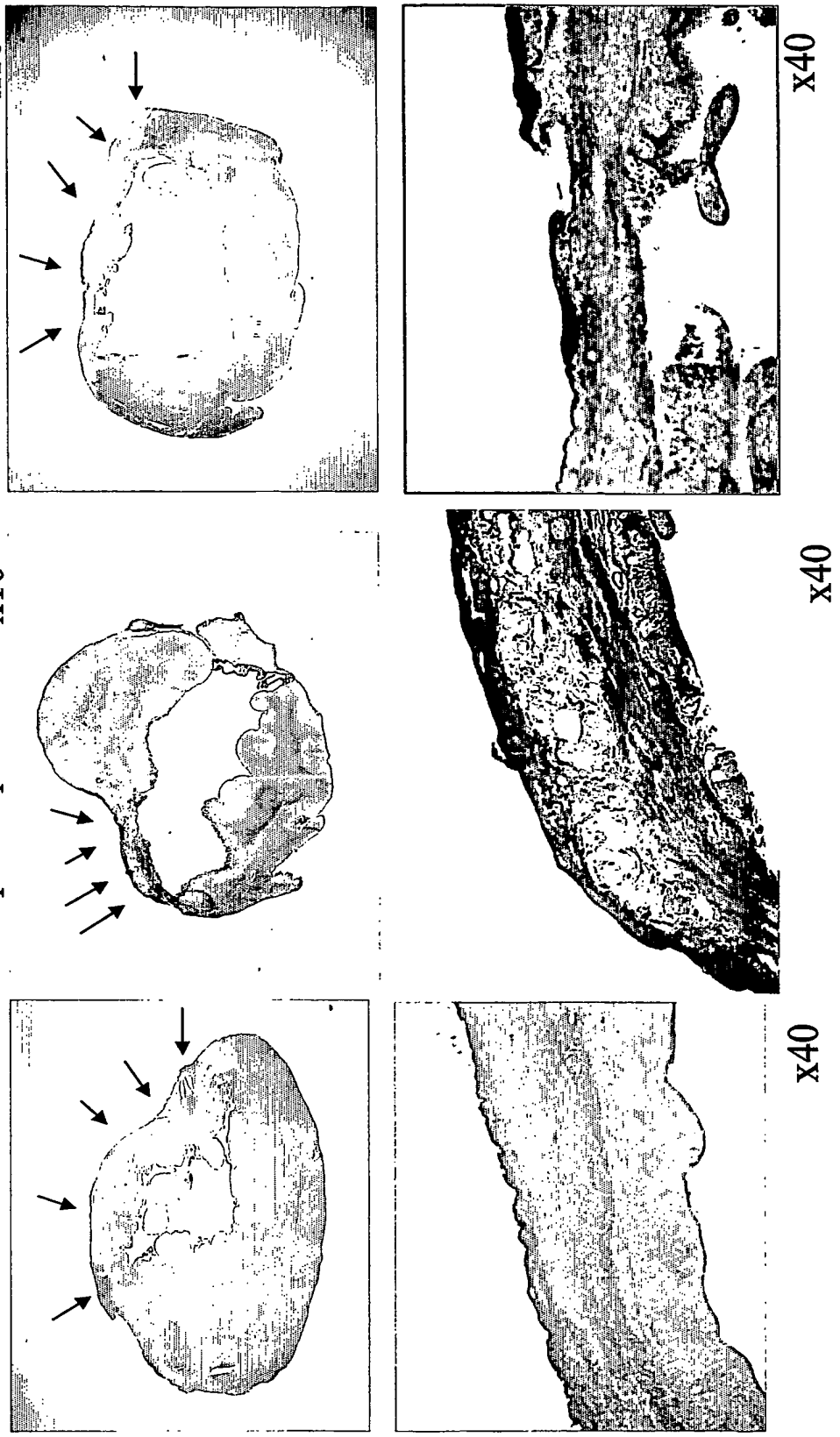

HE staining x400
MHC slow x400
Masson's Trichrome staining x400
MHC fast x400

FIG.30A

Tissue (Masson's Trichrome staining)



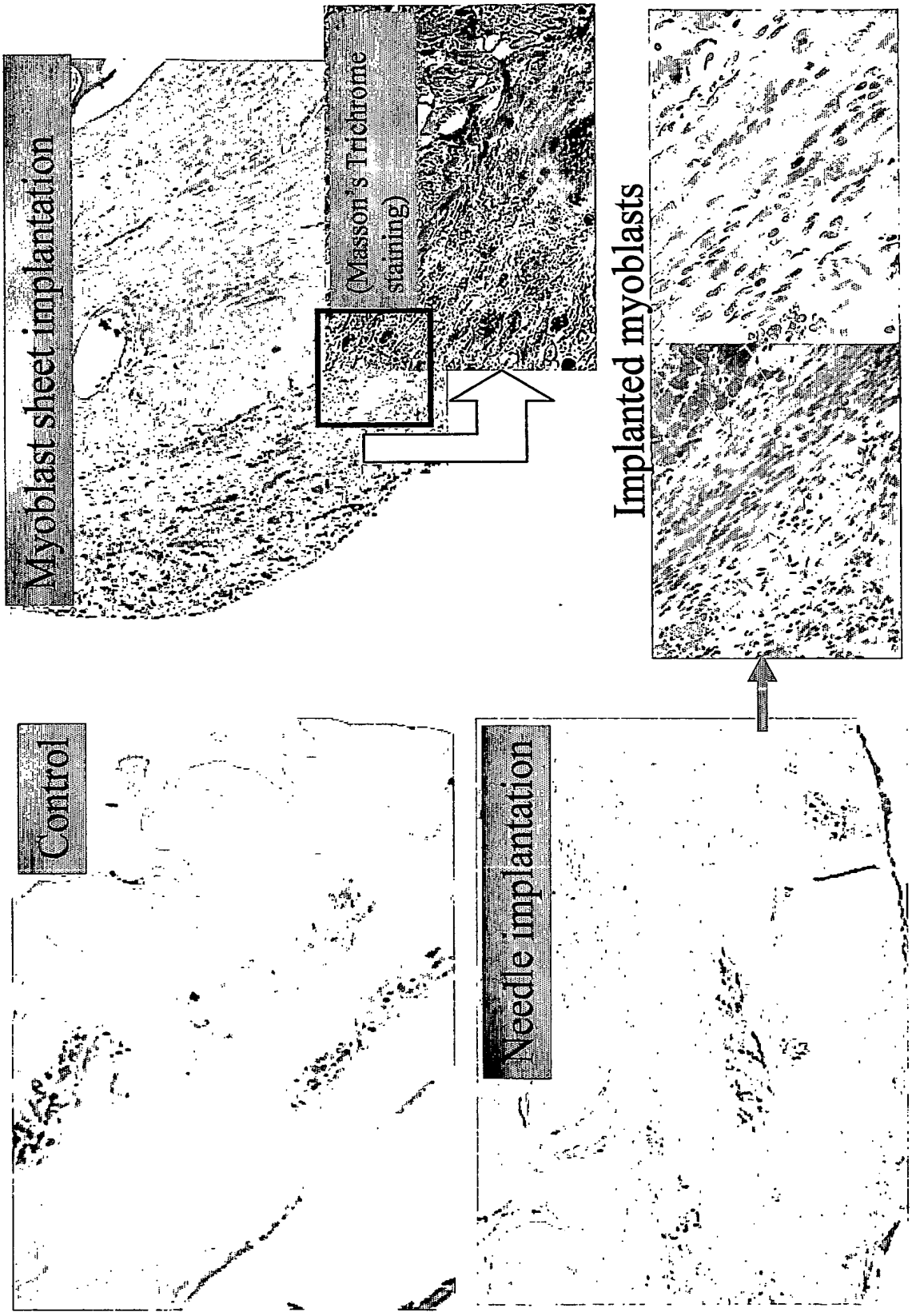

Myoblast sheet implantation
(Masson's Trichrome staining)
Implanted myoblasts
Control
Needle implantation

FIG.30B

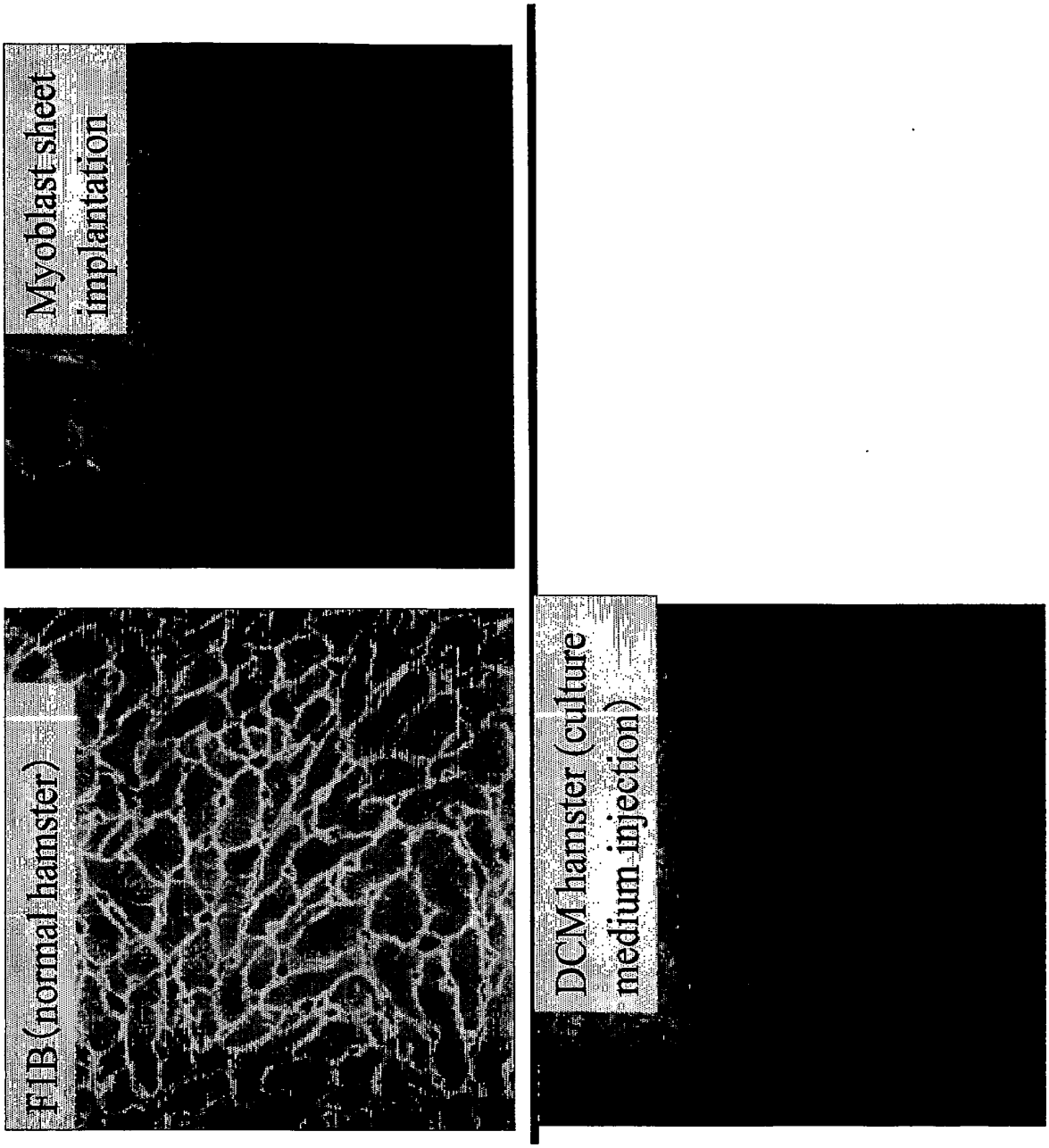
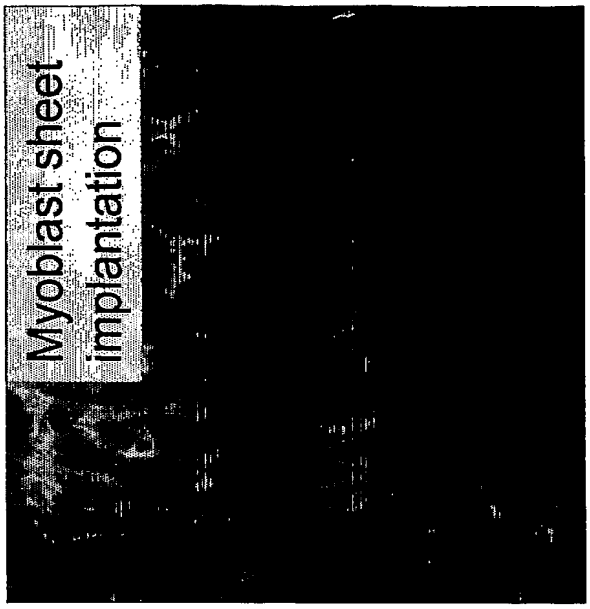
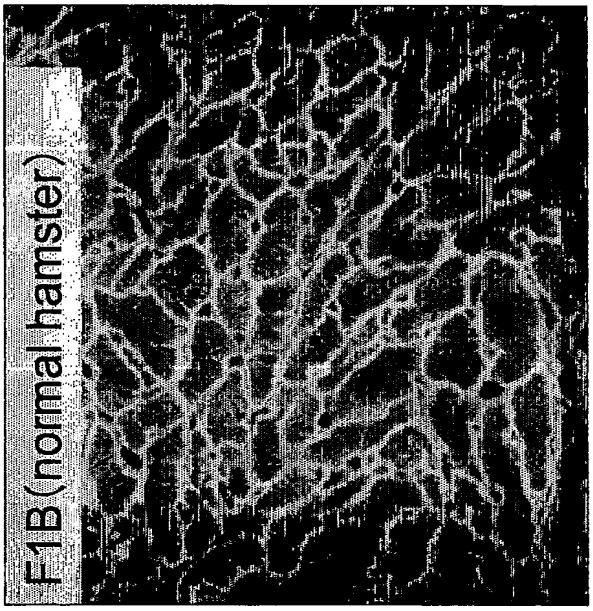

Myoblast sheet implantation
F1B (normal hamster)
DCM hamster (culture medium injection)

FIG.30C


Myoblast sheet implantation


F1B (normal hamster)


DCM hamster (culture medium injection)

FIG.30D

FIG.31

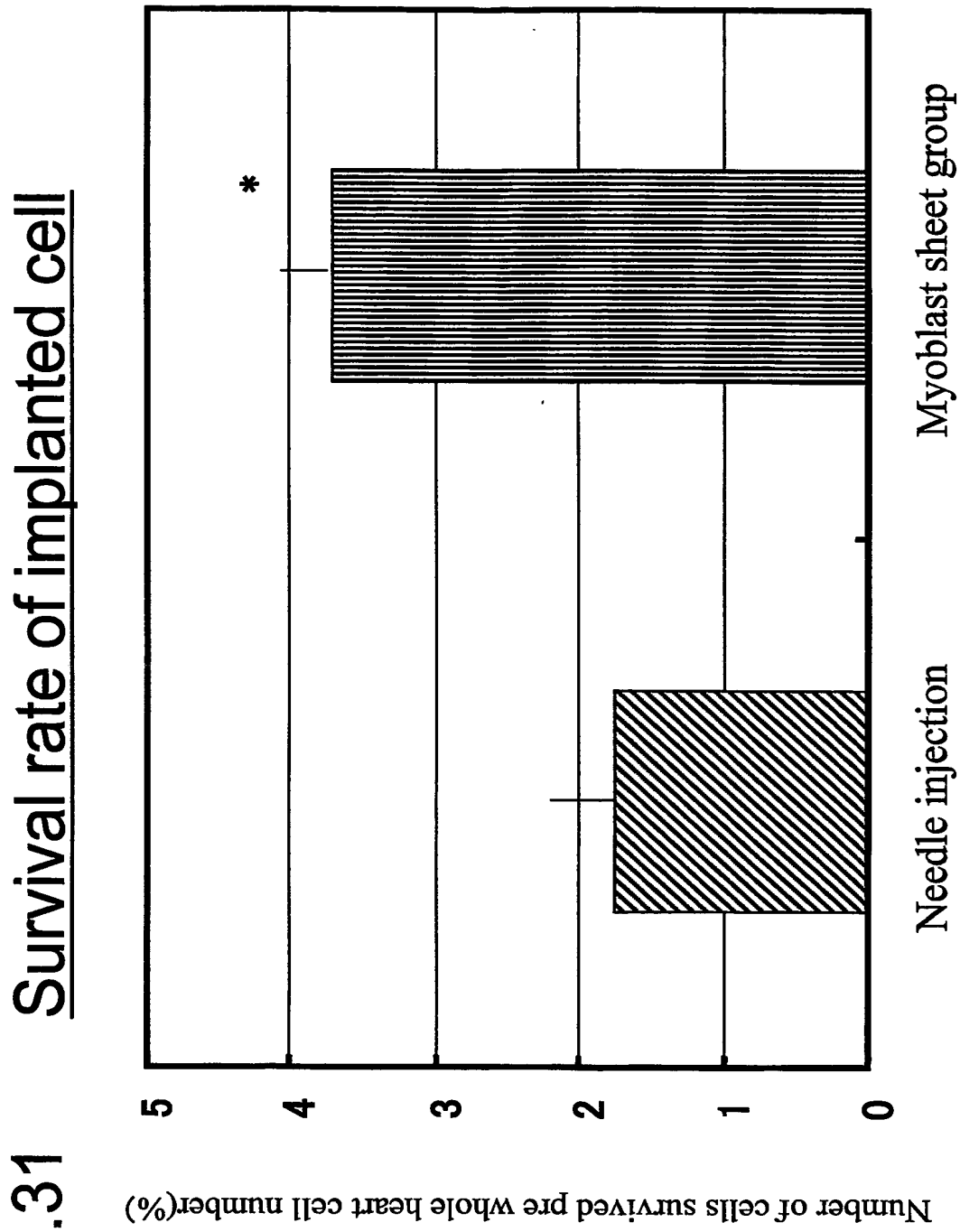

Survival rate of implanted cell
Number of cells survived pre whole heart cell number(%)
5
4
3
2
1
0
*
Myoblast sheet group
Needle injection

FIG.32 Electrorical propertes of myoblast sheet

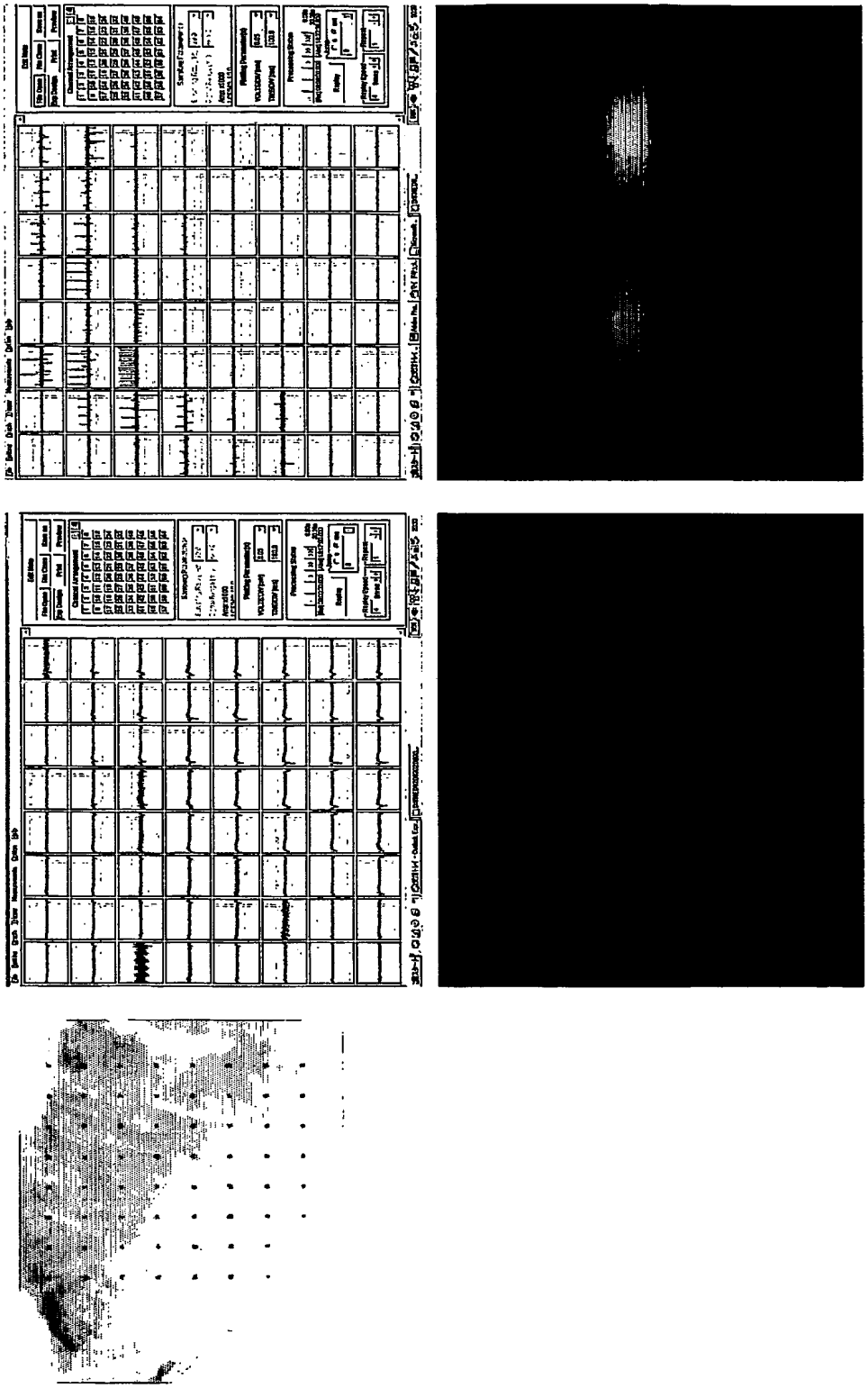
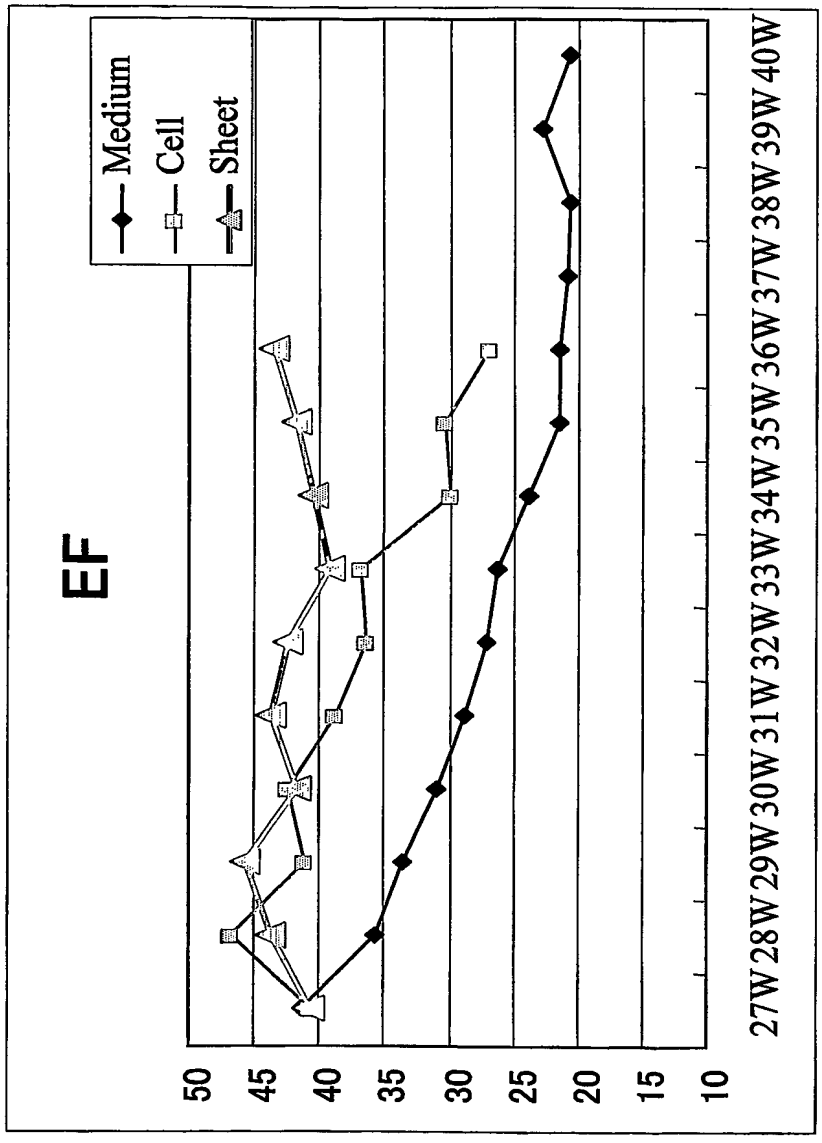
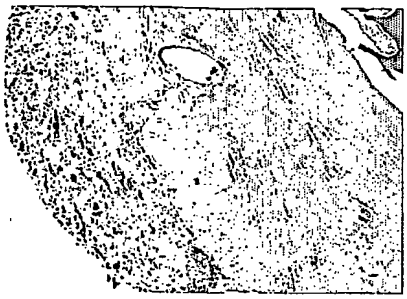


FIG.33A Myoblast sheet implantation to dilated cardiomyopathic hamster



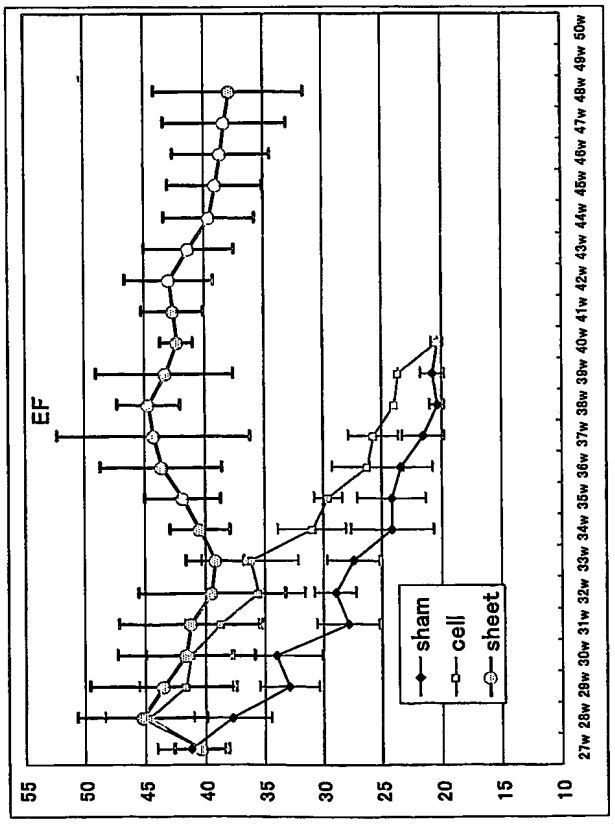
HE staining



Masson's Trichrome staining

FIG.33B

Left ventricular end-systolic diameter



Left ventricular end-diastolic diameter

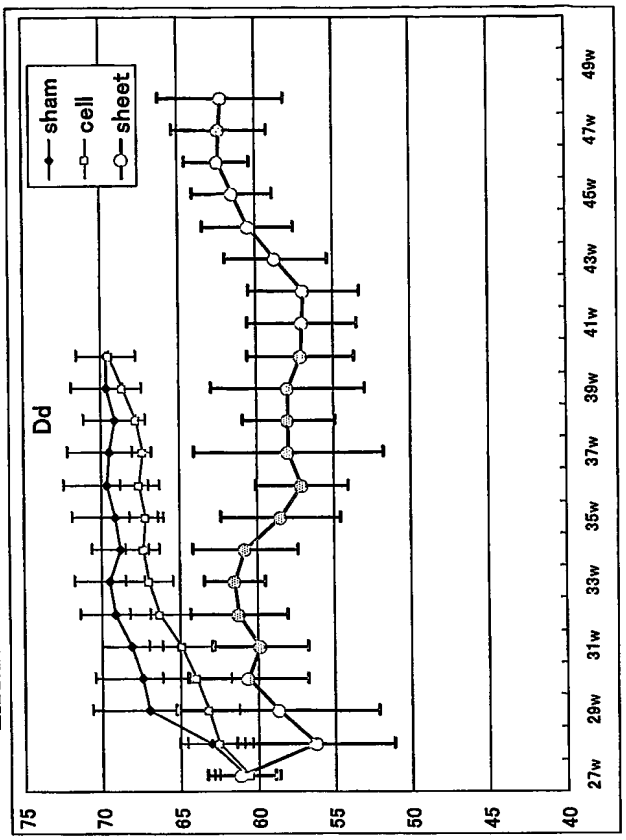
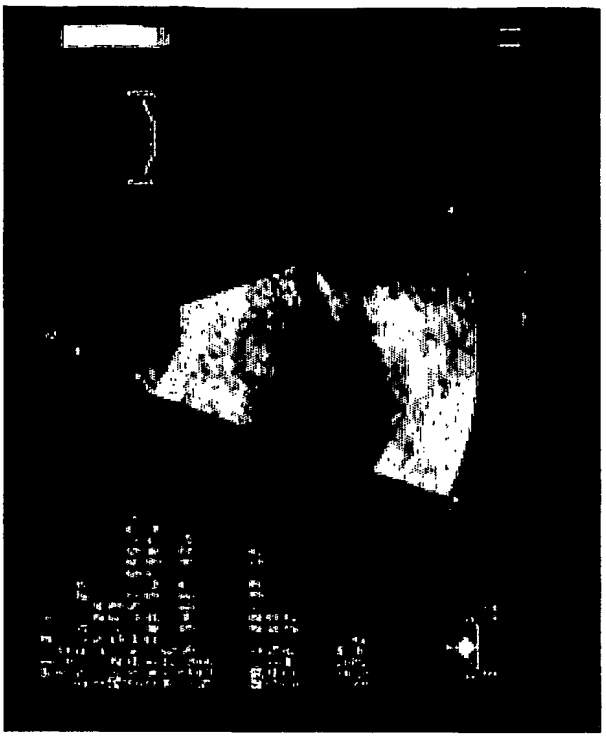


FIG.33C

Myoblast sheet implantation group



Control group

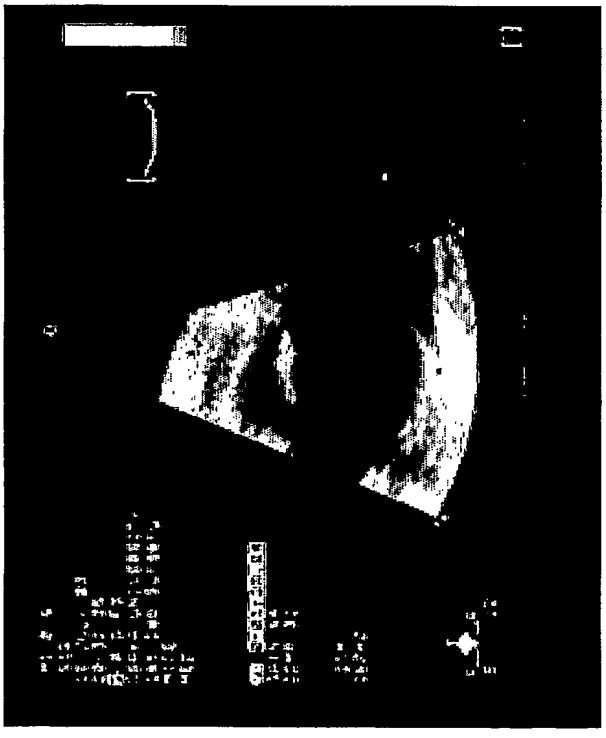


FIG.34 Myoblast sheet implantation into pig infarction model

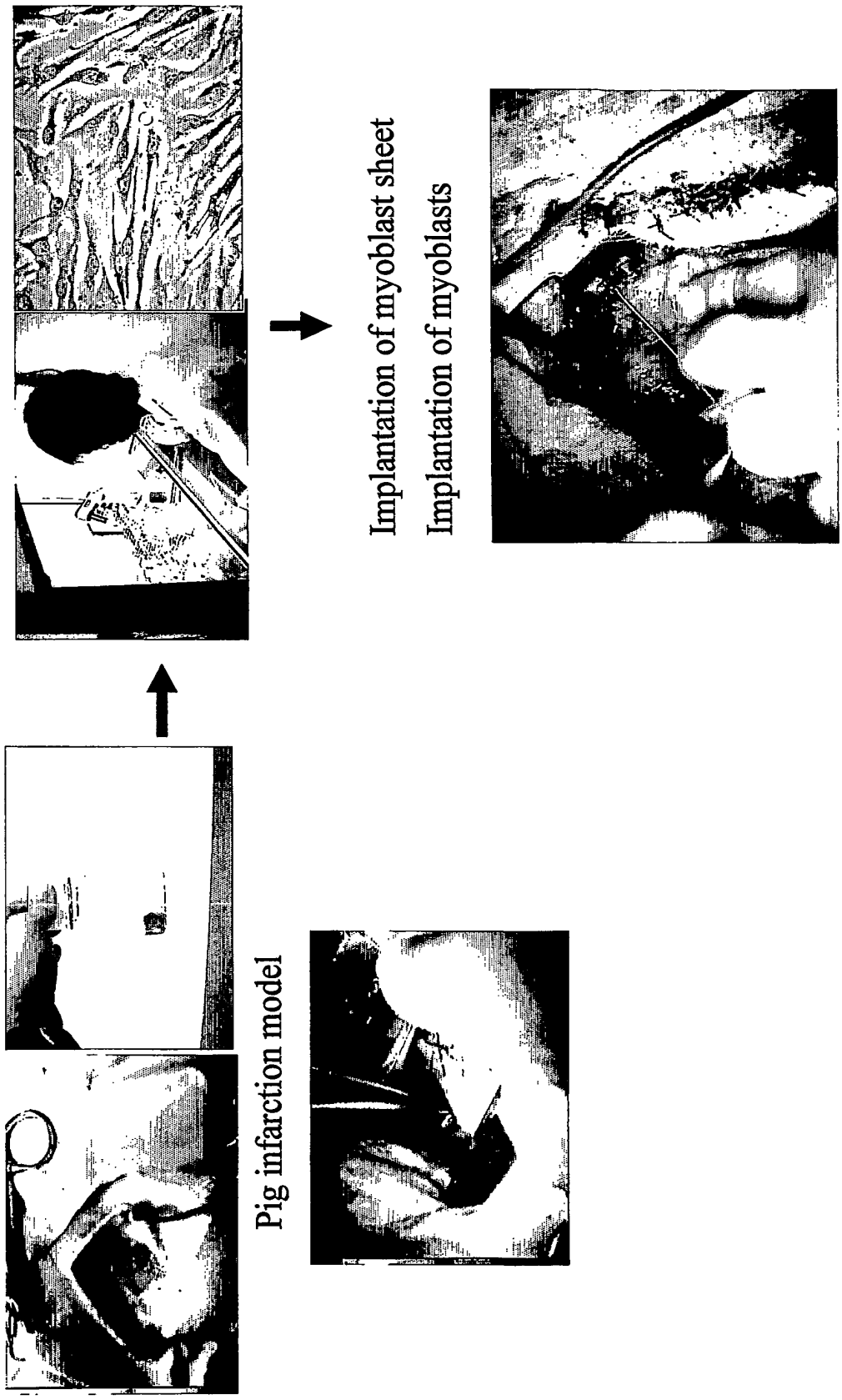
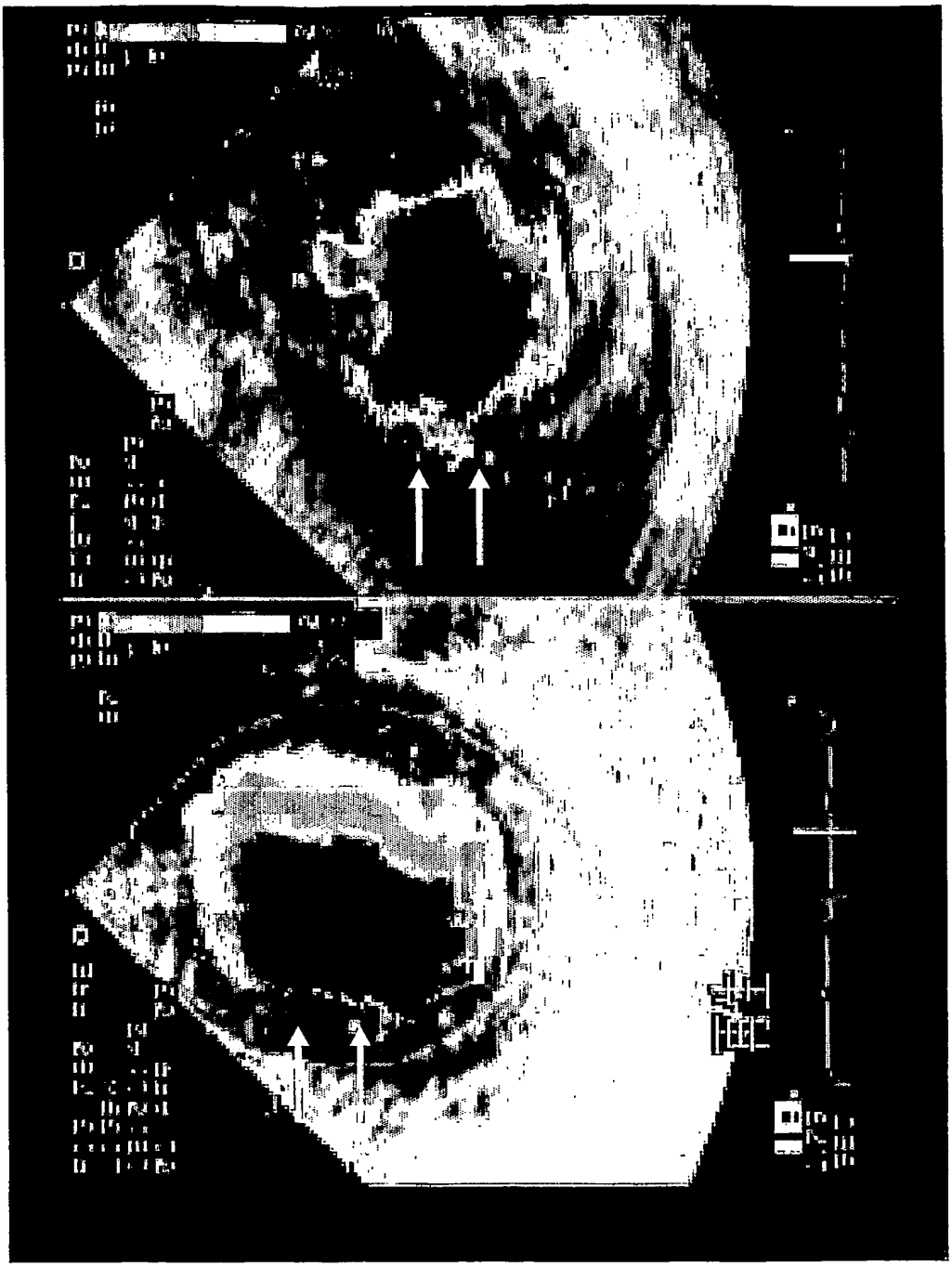


FIG.35

Evaluation of cardiac function (systolic function) of pig infarction model by CKI method

Before operation

After operation



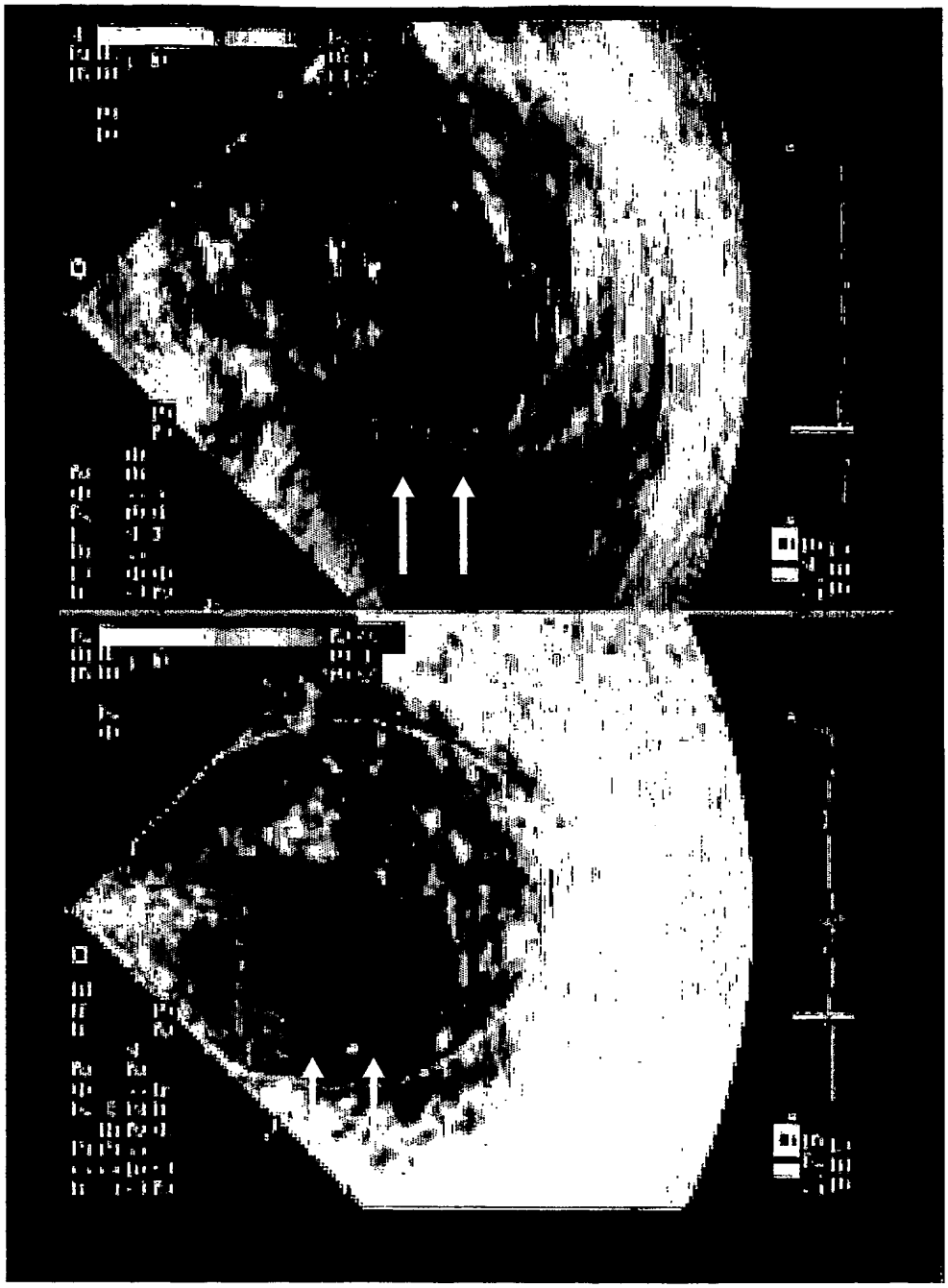
Implantion site

FIG.36

Evaluation of cardiac function (diastolic function) of pig infarction model by CKI method

Before operation

After operation



Implantion site

FIG.37

Without ascorbic acid

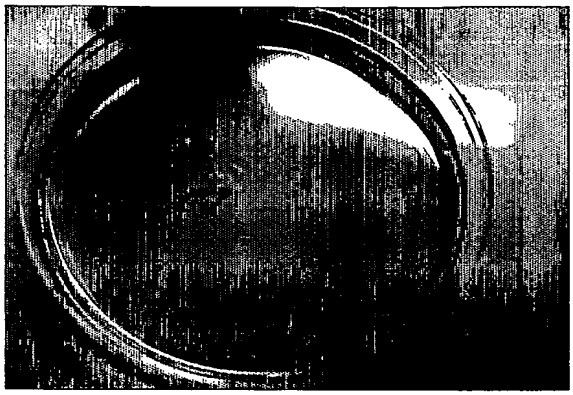


FIG.38

With ascorbic acid

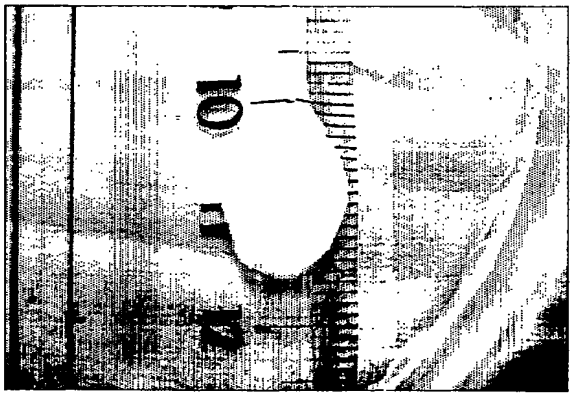


FIG.39

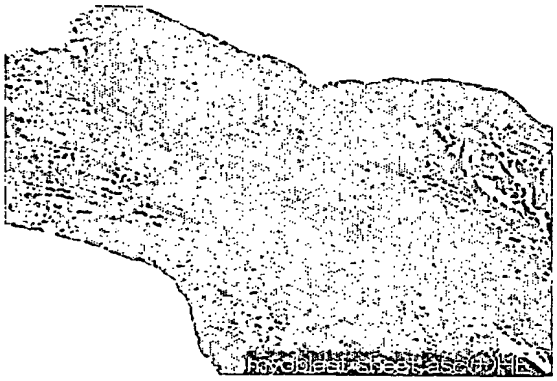


FIG.40

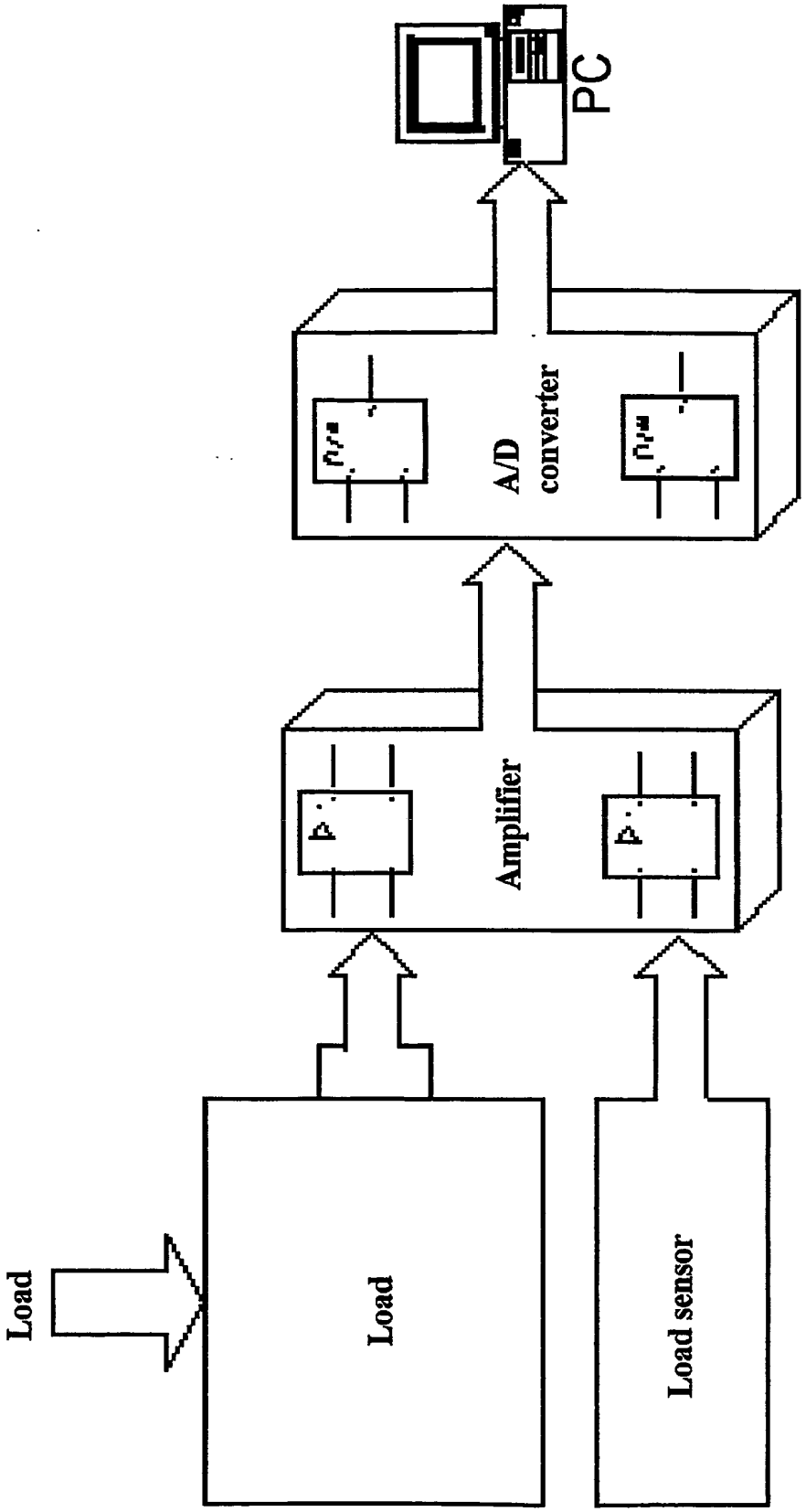
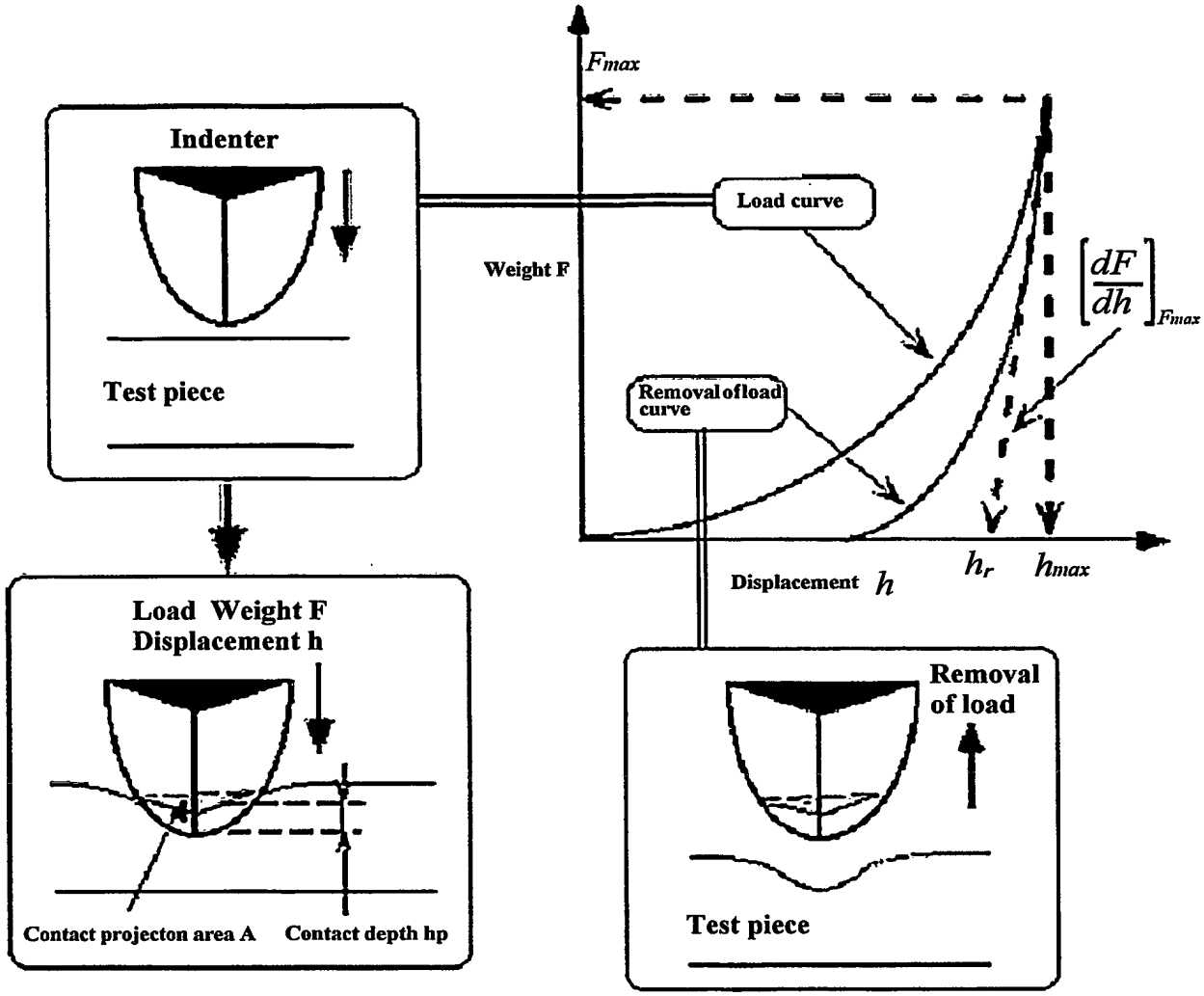


FIG.41



Rigidity $H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$

Young's modulus $E = \left[\frac{dF}{dh}\right]_{F_{max}} \frac{1-\nu^2}{2 \cdot k_2 \cdot h_{pmax}}$

Contact depth $h_p = h_r + 0.25(h_{max} - h_r)$

F : Load
A : Contact projection area
h_p : Contact depth
$k_1 k_2$: Shape coefficient
F_{max} : Maximum load
h_{max} : Max. displacement
h_r : Point at which tangential line cross weight 0
dF/dh : Gradient of tangential line of the removal of load curve
ν : Poisson's ratio

FIG.42